PREVALENCE OF BABESIOSIS IN SANGA CATTLE IN THE OHANGWENA REGION OF NAMIBIA

by

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DECLARATION

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I declare that the above dissertation is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by me as complete references.

_____ _____

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DEDICATION

I dedicate this work to my parents (Mr Pennti Matheus and Mrs Ndarila Eeru) who from a very young age taught me to work hard. This is a sign that they were successful in their endeavours. I am grateful to my wife (Mrs Johanna Andjamba) and children (Pennti, Tuyambeka and Pombili) for their support and understanding through the tough stages of my life. May God bless you!

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ABBREVIATIONS

AHT- Animal Health Technician

CI- confidence interval

DVS- directorate of veterinary services

EDTA- ethylenediamine tetra-acetic acid

ELISA- Enzyme-linked immunosorbent assay

IFA- Indirect Fluorescent Antibody

NaCl- sodium chlorine

NCA- northern communal areas

PBS- phosphate buffered saline

Spp- species

SVO -state veterinary office

NamLits- Namibia Livestock Identification and Traceability System

Df- degree of freedom

ABSTRACT

Title: Prevalence of babesiosis in Sanga cattle in Ohangwena region of Namibia

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Bovine babesiosis is one of the common, economically important tick-borne infectious diseases of cattle. Clinical cases suspected to be babesiosis are frequently observed in the study area. Yet to date, no studies have been done in the farming areas of the north central of Namibia to establish the prevalence of the disease. The objectives of the present study were to determine the sero-prevalence of different *Babesia* species in Sanga cattle; determine the most prevalent *Babesia* species and the spatial distribution for two *Babesia* species in the study area. A total of 392 cattle were randomly sampled and bled to collect blood at various crush pens in all constituencies of the region. This was done during the annual vaccination campaign against lung sickness. The IFA (Indirect Fluorescent Antibody) a method known to have a sensitivity of 95% and specificity of 99%, was used to analyse the samples for *Babesia* antibodies. The most prevalent species was *Babesia bigemina* with an estimated prevalence of 36.5%, followed by *Babesia bovis* at 16.6%. Mixed infections were estimated to be 13.2%. The disease was found to be prevalent throughout the region with no significance association between infection as the dependent variable and independent variables like sex, age and place. The parasite was widely but not uniformly distributed in the study area. There is a need for more farmer education and awareness. The region proved to be endemically unstable for babesiosis, a vaccination protocol to establish good herd immunity is necessary to improve production. Similar studies in different parts of Namibia be performed and that efforts to prepare a local vaccine. Agricultural regulations should also include the removal of old animals to help improve productivity and farmers output.

Further research should investigate and map out the prevalence of Babesia parasites and other heamoparasites in each region of Namibia. This information can also contribute towards the development of future interventions and management strategies in animal health.

Chapter 1

General Introduction

Background

Tick-borne diseases are of economic importance due to direct and indirect impacts. Direct impacts include; losses due to death of the animal and lower production. While indirect impacts include effects of quarantine measures, cost of tick control, vaccination and purchasing acaricides or dipping to control ticks on cattle. The quarantining and spraying of thatch grass from Okavango region before it can be transported to the other parts of the country is a practical example of the importance of ticks and tick-borne diseases (Kamani et al. 2010; Bock et al. 2004).

Babesiosis is one of the most common and economically important tick-borne infectious diseases of domestic and wild animals. The disease babesiosis is caused various protozoon of *Babesia* species (Vos & Waal 2004; OIE 2013). These parasitic microorganisms are found in the blood plasma; they are transmitted to the host by ticks (heamoparasites) through blood sucking. Babesiosis like other tick-borne diseases are generally characterised by fever, anaemia, debility and emaciation (Penzhorn 2015).

The Sanga Cattle, a type of Zebus *(Bos indicus)* was brought to Africa during the Arab invasion of the continent. It arose as a result of crossbreeding between the original hump less cattle from North America and the short horned Zebu cattle. Its characteristics of heat tolerance, smooth coat, light colour, but full pigment helped the Sanga cattle survive and prosper in one of the hottest environments of Namibia (Bock et al. 2004; Olivia et al. 2015).

The Sanga cattle breed is the most common breed found in the Northern Communal Areas of Namibia. Other breeds such as Afrikaner, Brahman, and Simbra are also found in the area but are scarce. Sanga are more resistant to parasites and tick borne diseases, and have them, ability to reduce the number of ticks on their skin. This lowers the number of blood sucking parasite on the body (Estrada-Peña & Salman 2013; Torr et al. 2002).

In Namibia, the most important control measure used to prevent the spread of tick-borne diseases; is restricting the movement of thatch grass from Okavango region to the rest of the country as per Animal Health Act1 of 2011. The permit to move thatch grass across the

country is issued on condition that is treated with acaricides and quarantined under state veterinary supervision. On contrary, there are no measures that prevent animal movement so as to control tick borne disease (Republic of Namibia 2011). Information from the Northern Communal Areas regarding the prevalence of infectious diseases is often not available or is of a poor quality (Latif & Walker 2004; Pfitzer 2009; Robbins 2012). In view of this, there is a need to fill the knowledge gap.

Lack of infrastructures, education and unemployment rates might contribute to poor production and diseases control in the Northern Communal Areas of Namibia like is the case in other communal areas in southern Africa. Therefore unlike their counter parts, farmers in such areas fail to realise high production indicators such as calving rate, growth rate and herd mortality (Robbins 2012; Góes et al. 2008). Babesiosis inhibits animal production through retarded growth, low meat production and even mortality of farm animals (Robbins 2012; Góes et al. 2008; Gualito et al. 2012).

Clinical signs suspected to be associated with babesiosis have continuously been reported at the state veterinary offices. However, there is no evidence of studies that have been done in the study area to ascertain the level of prevalence of the disease. It is therefore hoped that information generated in this study will increase awareness of the veterinary services and farmers of the prevalence of babesiosis and its distribution in the study area. Findings of this study will also help farmers and stakeholders to understand the risk level of the disease in the study area (Republic of Namibia 2013) .

Problem statement

Conditions suspected of being transmitted by heamoparasites and verminosis are continuously reported in the Northern Communal Area (NCA) of Namibia (Republic of Namibia 2013). However, there are no clear records stating the prevalence of babesiosis in the region. Due to the economic significance of these diseases, there is a need to establish the true prevalence and distribution of the disease in the study area.

Hypothesis/Assumptions

It will be possible to demonstrate the prevalence and spatial distribution of babesiosis in the study area. It will also be possible to compare the prevalence of the two *Babesia* species in the area.

Purpose statement

The purpose of the study was to establish the sero-prevalence of *Babesia* species in Sanga cattle of Ohangwena region, as well as to compare the prevalence of the two *Babesia* species in the region.

Research questions

a. What is the prevalence of bovine babesiosis in Ohangwena region?

b. Do *Babesia* species exhibit a distinct spatial distribution in Ohangwena region?

c. Which of the two *Babesia* species is more prevalent in Ohangwena region?

Study aims and objectives

The aim of this study was to establish the sero-prevalence of *Babesia bovis* and *Babesia bigemina* in Sanga cattle in Ohangwena region, and to also demonstrate the prevalence and spatial distribution of the disease in the study area.

The objectives of the present study included the following:

i. To determine the sero-prevalence of babesiosis in Sanga in Ohangwena region.

ii. To determine the most prevalent of the two *Babesia* specie in Ohangwena region.

iii. To demonstrate the distribution of both *Babesia* species in the study area.

Anticipated benefits of the study

The outcome of this study has the potential to assist the government and farmers to make more informed decisions regarding the prevention and control of the babesiosis in the study area.

Information obtained from this study will be freely available to farmers, agricultural officials, researchers and wider scientific community requiring such information. Furthermore, data obtained will assist with addressing areas where there may be shortfalls and facilitate the provision of necessary advice by State Veterinary and Agriculture Extension Officers and Technicians. The government is striving to help farmers improve the market price of beef farming in the Northern Communal Areas hence the knowledge of disease prevalence is vital.

Ethical considerations

Cattle were restrained properly in the crush pen to prevent injuries during vaccination and sampling. Most cattle in Ohangwena region have electronic ear tags, blood was only collected from cattle with ear tags (ear tags have unique numbers for each cattle). During the course of the study, if cattle tested positive, the information was channelled to the owners through the State Veterinary Office (SVO) and advised to have the animals treated. The details of the research were explained to farmers prior to requesting for permission to sample the animals. This was done during the morning briefing. Participating farmers were requested to sign the consent form (Annexure III) to indicate that they had consented to participating in the study.

Ethical approval was acquired from the UNISA Ethics Committee (Annexure I: Ref. Nr.: 2013/CAES/147) before the study could commence.

Components of the report

This dissertation consists of 6 chapters:

Chapter 1: Gives the background, problem statement, hypothesis, purpose statement, research question, aims and objectives, anticipated benefits of the study and ethics consideration.

Chapter 2: Presents the literature review with particular reference to the topic.

Chapter 3: Outlines the research methodology applied.

Chapter 4: Presents the results obtained in this study

Chapter 5: Discusses the results obtained in conjunction with the literature review.

Chapter 6: Presents the conclusions and reflects on the objectives and provides recommendations.

Chapter 7: References from the literature read and used in the discussion.

Chapter 2

Literature Review

2.1 The disease bovine babesiosis

Babesiosis also referred to as piroplasmosis, texas fever, red water, tick fever, is a dangerous, invasive, febrile tick-borne disease of animals (Penzhorn 2015; Schischke 2015; Abdullah-Al-Mahmud M.D., Shariful Hoque Belal S.M. 2015; Bosman et al. 2013; Gohil et al. 2013).

The protozoan *Babesia* that parasitizes red blood cells includes *Babesia bovis*, *Babesia bigemina, Babesia divergens and Babesia major*. Among these four species *Babesia bovis* and *Babesia bigemina* are known to be the most important species causing bovine babesiosis in southern Africa (Da Silva et al. 2013; OIE 2012b; Bock et al. 2004). The disease babesiosis plays an important role in the livestock industry worldwide. The details on the causes, effects and control methods of the disease are the focus of this chapter.

2.2 General distribution of Bovine babesiosis

Bovine babesiosis occurs in all the continents of the world wherever vectors like *Boophilus decoloratus, Rhipicephalus evertsi* and, *Boophilus annulatus* exist. The disease prevails in tropical and subtropical areas (Ursula J. Blumenthal, Jay M. Fleisher 2001; Gray et al. 2010; Hesterberg 2007; Perez et al. 1994; Stevenson 2008).

The vectors for Bovine babesiosis have a global distribution, stretching from the polar circle to the equator (Vos & Waal 2004; Republic of Namibia 2013). In Namibia, tick fever is frequently reported in farmers' reports to veterinary offices throughout the region (Republic of Namibia 2013).

Generally *Babesia bigemina* and *Babesia bovis* have equal distribution in most areas of the world. However, in Africa *Babesia bigemina* tends to be more widespread than *Babesia bovis*. This could be due to the fact that *Babesia bigemina* has many vectors as compared to *Babesia bovis*. In northern Africa, *Boophilus annulatus* remains the main vector of *Babesia bovis* and *Babesia bigemina*. It is also the principal vector for both parasites in the Middle East, some areas of southern Europe and in the southern areas of the former USSR (Vos & Waal 2004; Penzhorn 2015).

Ixodes ricinus is the main vector for *Babesia divergens* and this probably limits its distribution into the northern Europe, Spain and United Kingdom (Kubelová et al. 2012).

Babesia bigemina and *Babesia bovis* and their vectors were once enzootic in most parts of the southern United States of America. However, they are currently found only in a quarantine buffer zone along the Mexican border. Studies have proven the existence for *B. divergens* throughout Europe, and it may also occur in North Africa. Its vector, *I. ricinus*, can survive from northern Scandinavia to the Mediterranean. However, because this tick requires 80% humidity, it can be found only in some microenvironments such as the base of vegetation in forests, rough hill scrub, and damp low-lying land. This explains why its existence in Namibia where the weather is hostile is limited (Hoch et al. 2012).

2.3 Clinical signs of babesiosis

Babesiosis is generally characterized by extensive erythrocytic lysis leading to anaemia, icterus, hemoglobinuria and mortality (Reginald De Deken, Ivan Horak, Maxime Madder 2014; Homer et al. 2000). However, the clinical signs of babesiosis vary with the age of the animal, the species and strain of the parasite. Farmers frequently detect hemoglobinuria as a first prominent clinical sign. Poor appetite and high fever may first appear before other signs. Because of haemolysis and anaemia that is associated with the disease, certain characteristic signs may appear like the mucous membranes appearing pale and jaundice may occur. Furthermore, the sick cattle may isolate itself from the rest of the herd; appear weak, depressed and reluctant to move. Pregnant cows may abort. In severe cases there is an acute onset of fever (up to 41°C), anorexia, depression, weakness. The cessation of rumination and an increase in respiratory and heart rate may follow (Da Silva et al. 2013; Lemma et al. 2015).

Other symptoms include, increased intestinal and ruminal motility, and pipe stem diarrhoea that occurs due to spasm of the anal sphincter. Meanwhile, parasitaemia may rise to between 30 and 45%. This leads to the extensive destruction of the erythrocytes. As the anaemia increases, the animal gets more depressed. Dehydration is severe, and constipation replaces diarrhoea (Smith et al. 2000; Suarez & Noh 2011; Homer et al. 2000).

When standing a few meters from the cow, a loud heart sound can be heard, which is a manifestation of a rapid heart rate. Hemoglobinuria ceases as the fever diminished below

37ºC. When the disease persists the animal becomes recumbent, exhibits toxaemic shock, temperature falls below normal, the pulse is weak, develops severe jaundice, constipation, and dehydration (Kubelová et al. 2012).

Death occurs as a result of cardiac failure or hepatic and renal insufficiency. Enlarged and darkened liver and kidney jaundice and a swollen spleen of a soft pulpy consistency may be observed at post-mortem examination. Ecchymotic haemorrhages may be seen under the epicardium and the endocardium, and the pericardial sac may contain a large quantity of blood-stained fluid (red water). The degeneration of hepatocellular and hepatic necrosis may also be observed (Carroll et al. 2014).

2.4 Epidemiology of babesiosis

Babesiosis affects cattle, horses, sheep, goats, pigs, dogs and wild animals. The infection is mainly transmitted by ticks of *Boophilus* species. Tick fever occurs in most parts of the world wherever the vector is present (Martinot et al. 2011; Amorim et al. 2014; Alam, T.H. and Nasr 2010). *Babesia bovis* and *Babesia bigemina*, the most common causes of bovine babesiosis in southern Africa, occur wherever *Boophilus* ticks are encountered (Tembue et al. 2011; Cammà C. , Maseke M. , Pascucci I. , Di Domenico M., Molini U. , Scacchia M. 2012).

As discussed in section 2.2 above, the disease babesiosis is prevalent throughout most tropical and subtropical regions which includes North and South America, Southern Europe, Africa, Asia, and Australia (Stevenson 2008; Alaa et al. 2011) .

Babesiosis is more common in the wet season when the tick vectors abound, but less common in dry weather or dry areas where the survival of ticks is limited (Gray et al. 2010; de Castro et al. 1997; OIE 2013). Cattle are the principal hosts of the disease, but some reports suggest that water buffalo and African buffalo may also be infected but may not be significant reservoirs of the parasite (Mahmood 2012).

Grazing together of animals susceptible to babesiosis such as goat and sheep might result in some becoming reservoirs of the infection of cattle and vice-verse since they share the same pastures and continue to infect the tick vector (Cantu-C et al. 2009; Barré et al. 2011).

2.5 Transmission of babesiosis

Babesiosis is transmitted by external parasites called ticks, which get infection from ingesting parasites in the blood of the infected host. The vector ticks for *Babesia bigemina* are *Rhipicephalus microplus* (formerly *Boophilus microplus*) and *R. annulatus* (formerly *Boophilus annulatus*). However, *R. decoloratus, R. geigyi,* and *R. evertsi* can also transmit this species (Barré et al. 2011; Ayoob et al. 2010). On the other hand, *Babesia bovis* is transmitted mainly by *R. microplus* and *R. annulatus*. However, it can also be transmitted *R. geigyi* (Martin et al. 2011; Schnittger et al. 2012).

Other species which are not common in Southern Africa include; *B. divergens, B. jakimovi, and B. major* and *B. occultans*. *Babesia divergens* is transmitted mainly by *Ixodes ricinus, while Babesia jakimovi* is thought to be transmitted by an *Ixodes* species. *Haemaphysalis punctata* transmits *B. major*, *Haemaphysalis longicornis* transmits *B. ovata,* and *Hyalomma marginatum* transmits *B. occultans* (Martinot et al. 2011).

Other than through ticks, babesiosis can also be transmitted iatrogenicalyenically through direct inoculation of blood, and through biting flies and to a less extent by fomite (Ekici & Sevinc 2009; Sandra Ríos-Tobón , Lina A. Gutiérrez-Builes 2014).

2.6 Diagnoses of babesiosis

The diagnosis of tick-borne diseases is based on associating factors such as clinical signs, herd history, presence of the vectors etc. (Muhanguzi et al. 2014; Haapasalo et al. 2010). Non immunologic methods such as blood smear are also regularly used in the diagnosis of babesiosis, but limited to early infection or after the establishment of the carrier state in the animal due to low circulation of the parasite in the blood (Amorim et al. 2014; Nair et al. 2013).

According to the OIE (2012b) and Wright (1990) , immunologic methods that can be used to diagnose babesiosis include: enzyme-linked immunosorbent assay (ELISA) and Indirect Fluorescent Antibody Test (IFA) provided that the blood is collected into ethylenediamine tetra-acetic acid (EDTA) tubes. According to Bock et al. (2004) and Olivia (2015), serology test reflects the exposure of animal to the parasite but not necessarily indicative of the current

infection. Tests that are currently in use to detect the presence of antibodies for *Babesia* Spp. in blood have been described in details by Ngeranwa et al. (2008).

Studies by Penzhorn (2015) ; Ekici & Sevinc (2011)and OIE (2012a) indicated that, serological tests like IFA and ELISA are the recommended tests for epidemiological surveys and export certification due to their high specificity and sensitivity. Of the two tests, IFAT is the most common serological technique used to distinguish *Babesia* Spp. and also demonstrate the presence of *Babesia* antibodies in a given population (Terkawi et al. 2011).

According to Nair et al. (2013), IFA, ELISA and PCR are good for identify blood parasites for surveillance and export certification purposes. Ekici & Sevinc (2011), recommend the use of the ELISA and IFA diagnostic technique for research purposes because they give quantitative results. Blood smear that is widely used in clinical cases is not recommended for surveillance and hence it is not conducive for conducting surveys.

In view of the facts discussed in the paragraphs above, the researcher decided to use the IFA method to detect antibodies for *Babesia bovis* and *Babesia bigemina* as described by several other authors (OIE 2013) .

2.7 Treatment of babesiosis

The uses of diminazene aceturate and imidocarb dipropionate have been reported to be effective in treatment of babesiosis. However, the use of oxytetracycline together with imidocarb has also proved effective as treatment for babesiosis (Atif et al. 2012).

Remedies such as quinuronium sulphate, amicarbalide and diminazene aceturate were used as babesicides for the treatment of bovine babesiosis in most European countries for many years (Mosqueda et al. 2012). However, in the 1970s a fourth babesicides, imidocarb diproprionate was introduced and it took over the market in most European countries. The reason why this drug was favoured over the others was because of its therapeutic utility and its effectiveness as prophylactic treatment at twice the therapeutic dose. However quinuronium sulphate and amicarbilide were later withdrawn from the market due to manufacturing safety issues (Akhter et al. 2010; Penzhorn 2015).

Side effects such as coughing, muscular tremors, salivation, colic, and local irritation at the site of injection may follow a high dose of imidocarb diproprionate. Furthermore, imidocarb

has been found to be the most toxic babesicide when given intravenously, intramuscular or subcutaneously (Shkap et al. 2005; OIE 2013).

Despite the bad side effects, imidocarb remains the only babesicide, which consistently clears the host off parasites. However it is slower in action as compared to quinuronium sulphate (Barré et al. 2011; Atif et al. 2012; Vial & Gorenflot 2006). At the later stage, imidocarb can lead to parasites acquiring resistance because they can survive at low levels of babesicide for a long period. Furthermore, it has been reported that factors such as mineral deficiencies, changes in parasite virulence and host susceptibility may contribute to the parasitic resistance to drugs (Bock et al. 2004; Schnittger et al. 2012).

In order to facilitate the establishment of immunity, a certain period of antigenic exposure is necessary before the treatment (Dumler et al. 2001; Gualito et al. 2012). This could explain why cattle treated with imidocarb diproprionate, which is known to clear the host of parasites; tend to end up with a solid sterile immunity (De Vos & Bock 2000; OIE 2013).

In acute cases of babesiosis, blood transfusion is recommended as supportive therapy. However if transfusion therapy is given too late it could lead to treatment failure. Factors such as mineral deficiencies, changes in parasite virulence and host susceptibility may contribute to the parasitic resistance to drugs (Drummond et al. 2003; Suarez & Noh 2011).

2.8 Control of babesiosis

The OIE (2013), recommends three methods to control babesiosis, and these are: vaccination, chemotherapeutic regimes and tick control.

Vaccination is only used to control babesiosis but cannot eliminate the disease. Here live frozen or chilled vaccines against *Babesia bovis* and *Babesia bigemina* are used. It is done by subcutaneous injection of the live vaccine at an amount of 2ml per cattle. The form of preparation of these vaccines depends on the demand, transport networks and the availability of liquid nitrogen or dry ice supplies (Gualito et al. 2012).

According to Minjauw & Mcleod (2003b) and Gualito et al. (2012), frozen vaccine preparation provides room for a thorough post-production testing of each batch. However, the shelf-life of these vaccines is reduced once defrosted. Furthermore, the production cost for the vaccines is high and they are very difficult to transport. Blood-derived vaccine poses the

potential risk of contamination, therefore pre- and post-production quality control is essential. However, this leads to high production cost which renders the vaccines unaffordable to some countries in endemic regions (Góes et al. 2008; Reginald De Deken, Ivan Horak, Maxime Madder 2014).

Chemoprophylaxis as a control strategy provides protection to animal from clinical disease for three to six weeks but allows a sufficient level of infection for immunity to develop. However for chemoprophylaxis to be successful, the following conditions must be met (De Vos & Bock 2000; OIE 2013):

- the host should be guaranteed to contract babesiosis during the period of protection;
- the animal should acquire the immunity which smoothly turns the animal to a resistant state without an intermediate clinical stage

In endemic areas, integrated control measures are more appropriate. This involves first grazing the area with sheep that are resistant to *Babesia bovis* and *Babesia bigemina* infection. This should be done in the absence of cattle for a considerable period. This is then followed by the application of synthetic acaricides to cattle using slow-release devices or as pours-on acaricides. Acaricides with a high residual activity should be used during the periods of greatest exposure to tick vectors. The enzootic stability should also be maintained through assuring the continuous contact of cattle with vector tick and the parasites. Chemoprophylaxis and vaccination can be integrated to ensure that cattle become infected and immunized with minimum pathological effects (Estrada-Peña & Salman 2013).

Eradication of tick borne diseases is only achieved through the eradication of the tick vector and/or intensive chemotherapeutic regimes. In the past to date the use of acaricides to eliminate ticks served as a good measure to prevent haemo-parasitic diseases (Barré et al. 2011; Smith et al. 2000; Callow et al. 1997). Long term control programs and diligent surveillance should be implemented to prevent re-infestation and eradicate the disease (Atif et al. 2012; OIE 2013).

2.9 The prevalence of bovine babesiosis

In a sero-prevalence study done on cattle in Malaysia by Rahman et al. (2010) the results revealed that 17.0% were positive for *Babesia bovis*, 16.0% for *Babesia bigemina*, and 9.0% for both *Babesia bovis* and *Babesia bigemina* infections. Rahman et al. (2010) also

conducted a survey on cattle in Malaysia in 2009, and observed a prevalence of 17% for *Babesia bovis* and 16% for *Babesia bigemina* respectively. In Brazil one study estimated the prevalence of babesiosis to be at 63% for *Babesia bigemina* and for 18% *Babesia bovis* (Canever et al. 2014).

In Angola a study conducted by Kubelova et al. (2012) estimated the prevalence of *Babesia* species to be at 16% . In Nigeria, the prevalence of babesiosis (*Babesia bigemina and Babesia bovis*) as a single infection was found to be 63.0% of the positive samples (Kamani et al. 2010).

Meanwhile, sero-prevalence studies that were done on cattle in the eight provinces of South Africa using two different diagnostic methods (ELISA and IFA) to determine the serological prevalence of *Babesia bovis* and *Babesia bigemina* showed that 35.3% (ELISA) and 39.7% (IFA) of cattle surveyed were positive for *Babesia bovis.* Meabwhile 30% (ELISA) and 36.5% (IFA) were positive for *Babesia bigemina* antibodies. The prevalence for mixed infections was observed to be at 18.2% and 26.3%, respectively (Terkawi et al. 2011).

2.10 Socio-economic impact of babesiosis

Bovine babesiosis or tick fever is economically one of the most important tick-borne, parasitic infections that cause significant morbidity and mortality in cattle worldwide (Hangara et al. 2011; Homer et al. 2000). In Namibia, it has been reported that the socio-economic loses from these two organisms due to death and reduced production of cattle can be considerable and poses serious threats to communal farmers (Hangara et al. 2011).

In South Africa the disease is reportedly responsible for several deaths of cattle resulting in large loses for poor farmers (Mekonnen et al. 2002; Schischke 2015). According to Mylonakis (2001), lack of surveillance and asymptomatic infections make it difficult to estimate the prevalence of babesiosis. Even though economic loses has been encountered, precise estimates have not be established because it requires a large amount of information obtainable at a high cost. Economic evaluations need sensitive veterinary research data. Babesiosis and other helminths hamper rural development programmes and thus retard the economic growth in some parts of Africa (Baumgärtner & Tikubet 2012; Sikhweni, N.P. & Hassan 2013).

Ticks, as vectors for babesiosis cause substantial economic losses in cattle by reducing productivity and fertility, and sometimes causing deaths. The infestation of cattle with ticks reduces the productivity of cattle in a number of ways, including:

(i) the direct effect of attachment and feeding retards the growth and weight gain through malnutrition. (ii) Ticks inject the toxins in to cattle causing disease resulting in the cost incurred through veterinary consultation and treatment. (iii) Damage to the hides due to tick bites, which diminishes value for money at tanneries. (iv) A reduction in weight gain due to the sucking of blood by female, adult ticks (e.g., Rhipicephalus microplus). (v) Reduced milk production, and quality. (vi) Morbidity and mortality associated with the disease (Gohil et al. 2010; Mosqueda et al. 2012). All these losses pose a direct impact on food security. In addition, costs due to babesiosis are also incurred through its impact on international trade of cattle as a preventive measure on trans-boundary animal diseases (Minjauw & Mcleod 2003a; OIE 2013).

2.11 Public health aspects of babesiosis

Babesiosis in man has been reported in some countries where blood donors tested positive for *Babesia* antibodies (Senanayake et al. 2012). It has also been proved that *Babesia* organisms can be transmitted to man, making it a zoonotic disease. In man, it causes severe illness and can be fatal. Like in cattle, the disease is manifested by acute onset of severe haemolysis, hemoglobinuria, jaundice, persistent high fever, chills and sweats, headache and abdominal pain. Some patients do vomit and develop diarrhoea (Schnittger et al. 2012; Vannier et al. 2008). The mortality rate is high and is estimated to be 40% with antiparasitic remedies and supportive therapy. In view of this, when visiting endemic areas, it is advisable for people to take preventive measures such as the use of tick repellents and wearing long-sleeved shirts and long pants to prevent exposure to ticks (Oteo & Estrada 1992; Hoch et al. 2012).

Babesia species well known for their zoonotic nature include *B. microti and B. divergent*. They cause serious sickness in human and if not treated at early stage may result in death. Immuno-compromised people are particularly at a higher risk of contracting babesiosis. *Babesia divergens* also causes serious disease in humans who have had splenectomy (Wormser et al. 2006; Senanayake et al. 2012).

Although infection in humans is rare, as of 2003 about thirty cases had been reported in Europe. Therefore, to prevent infection with *B. divergens*, it is advisable that immuno-compromised people especially should be cautious when visiting babesiosis endemic areas, more so during the rainy season. According to Robbins (2012), skin and clothing should be inspected for ticks after a visit to the outdoors, and any ticks found should be removed. *Babesia bovis* may also be zoonotic, but this is uncertain. According to Vos (2004) and OIE (2013), some historical cases attributed to *Babesia bovis* could have been caused by *B. divergens*.

Chapter 3

Research Methodology

3.1 Materials and methods

In this section, the study area and the methods and materials used in the study are described.

3.1.1 Study area

Ohangwena is one of the thirteen regions of Namibia. The western part of the region has higher human and farm animals population than, the eastern part which is preferred for cattle posts. The region lies on the northern border of Namibia with Angola. Ohangwena borders with Cunene province in south and Cuando Cubango province in the far northeast of Angola. Domestically, it borders with Okavango to the east, Oshikoto to the south, Oshana to the southwest and Omusati to the west. It is traversed by the north-westerly line of equal latitude and longitude (Republic of Namibia 2015).

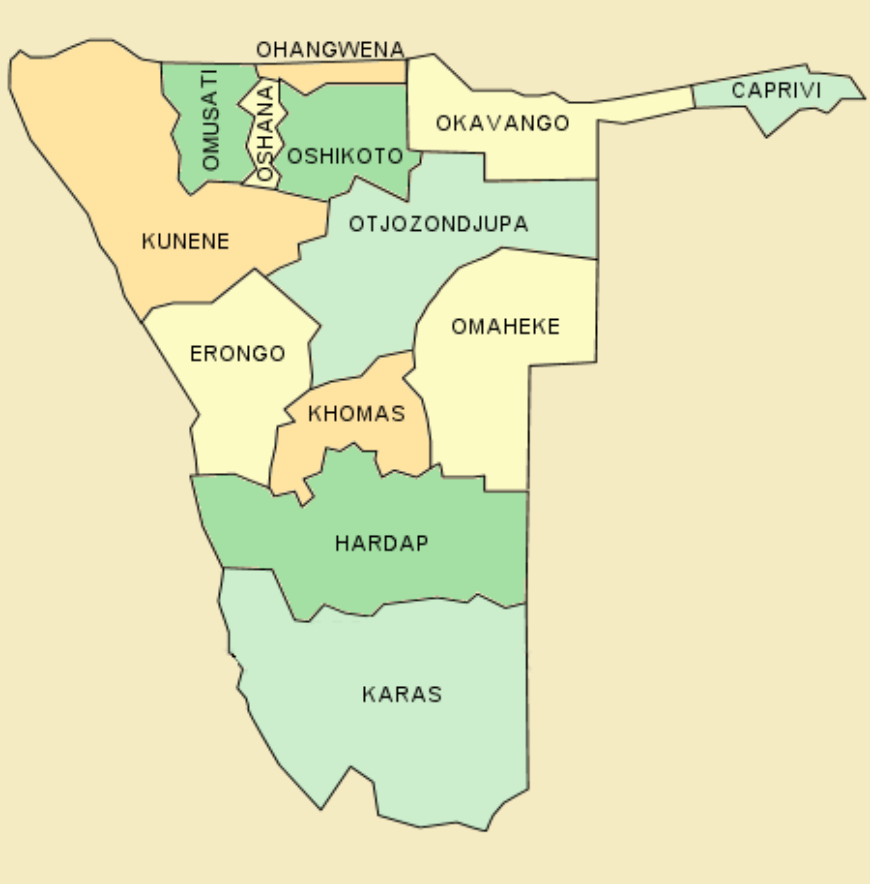

Figure 3.1: The map of Namibia indicating the study area Ohangwena (Republic of Namibia 2015)

The vegetation in the study area is characterized by semi-arid land with deep sandy soil without surface water, except for temporary flood pans linked to Cuvelai stream from Angola. Agro-silvi-pastoral (crop and animal farming together) system is the main farming activity, with the seasonal rain fed cultivation of pearl millet as grain for staple food. Even though the soil is suitable for crop production, the vegetation is suitable for livestock production (Republic of Namibia 2015). The average annual rainfall is about 450 mm per annum.

Based on the animal census of 2012, the cattle population was estimated to be about 200 000 cattle (Republic of Namibia 2013).

The tick infestation tends to be high during the rainy season and that is when seasonal flooding occurs. However, tick infestation remains a problem throughout the year, as per the veterinary office case registers (Republic of Namibia 2013).

Farmers in the Northern Communal Areas of Namibia have limited understanding of issues of animal disease control in relation to beef marketing (George et al. 2004; Sikhweni, N.P. & Hassan 2013; Mekonnen et al. 2002).

Farming cattle is mainly on a subsistence level, with cattle kept for social customs such as weddings and other cultural purposes. The Animal Health Act 1 of 2011 restricted the movement of animals from the north to the south of the veterinary cordon fence in Namibia.

The most commonly farmed breed of cattle kept by farmers in northern Namibia is the Sanga breed. However the prevalence of babesiosis in these cattle is not known as there has not been any studies to establish prevalence.

3.1.2 Research design

An observational cross-sectional study design aimed at determining the prevalence of babesiosis in Sanga cattle was used in the present study. According to Olsen (2014) and Owens (2002) a cross-sectional survey provides a snapshot of what is happening at that point in time from a sample selected to represent a larger population, and is good for estimating the burden of disease at a specific period of time. Based on this, a cross sectional study design was considered suitable for this study.

3.1.3 The sample frame and determining the sample size

According to Vetter (2012) and Canever (2014), the sample size increases as the population increases at a diminishing rate and remains relatively constant at slightly more than 380 cases. As per livestock census of three previous years, the cattle population in the study area is estimated to range from 175 000 to 200 000 cattle. Based on this report, the Figure 3.2 shows how the sample size was determined.

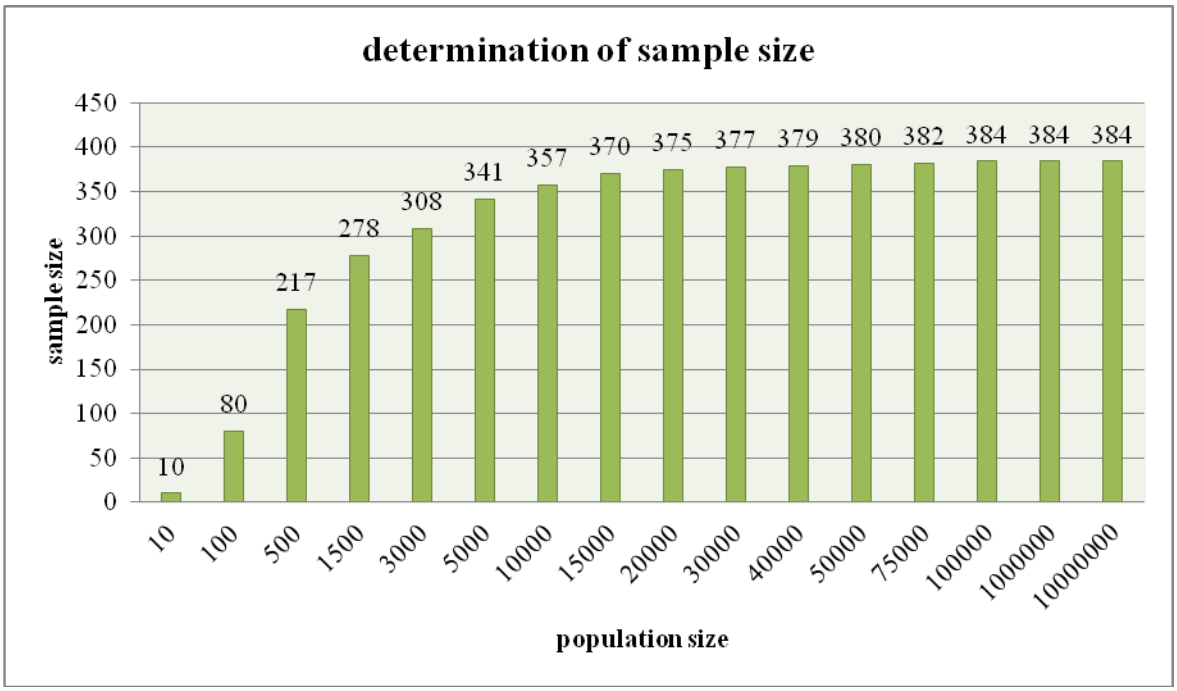

Figure 3.2: Chart for determining sample size (Fosgate 2009; Stevenson 2008; Muhanguzi et al. 2014)

Based on Table 3.3, the recommended sample size was estimated to be 384 blood samples. According to Thrusfield (2005) and Muhanguzi (2014), sample size in prevalence studies can also be determined using the following formula.

$$\mathbf{n = 4\ P\ Q/L^2}$$

Where:

n = sample number

P = estimated prevalence = 50%

Q = 1-P

L= range of precision desired, usually an estimate that is within 5% (0.05) of the true prevalence.

The number 4 represents the confidence interval, which is z^2 at 95% level of confidence that gives you a confidence interval of +/-5%, + or – 1.96 because the researcher wanted to be 95% confident that half of the cattle population in the study area might be infected.

Based in the calculations, it was estimated that 4 (0.5x 0.5)/0.0025 = 400 blood samples were required for this survey.

The author however, decided to use both the formula by Thrusfield (2005) and the method described in the Figure 3.3. From the two methods, it was estimated that the mean sample size of the two methods (384 +400/2= 392) be adopted for the required sample size for the study area.

3.1.4 Sampling strategy and sampling procedure

There were two hundred and fifty seven (n = 257) crush pens in the study area. Vaccination was carried out at five separate crush pens per day by five different teams. All teams were led by Animal Health Technicians (AHT). The researcher who was one of the five Animal Health Technicians carried out the sampling and vaccination simultaneously at crush pens every day.

Sampling of cattle was done during the annual vaccination campaign against lung sickness. This enabled the researcher to collect samples which represented the cattle population (approximately 175 000 – 200 000 cattle) in the area. The vaccination campaign was setup according to a predetermined programme that moved from one crush pen to the next in geographical order, which enabled the researcher to access forty nine (49) crush pens. Vaccination was done at five crush pens per day for about fifty working days. Therefore, sampling was done every day at one of the five randomly selected crush pens. The total number of crush pens (Figure 3.3) that were sampled was forty nine (49).

Figure3.3: Cattle restrained in the crush pen during sampling

During the sampling process eight (8) blood samples were collected from each of forty nine (49) crush pens. Proportional sampling was used to ensure equal representation of samples from the various constituencies.

Areas like Epembe and some parts of Okongo constituency that had no cold storage facilities for storing samples were excluded from sampling.

Systematic random sampling without replacements was used to sample cattle from which blood was drawn, so that every cattle in the study area had an equal chance of being tested. This was achieved by sampling every 8^{th} (eighth) cattle starting from the first 8th cattle that were determined randomly. Blood was then collected from every eighth cattle repeatedly or using a multiple of eight (8^{th}, 16^{th}, 24^{th} etc.). If the 8^{th} animal had no tag, the next animal was chosen.

Once an animal (cattle) had been selected as a sampling unit, it could not be reconsidered for sampling again. Therefore each animal had only one chance of being sampled (testing) to

avoid repeat sampling of animals. This helped to avoid bias associated with non-probability sampling techniques (Cameron 2012). Only cattle that had electronic ear tags qualified to be sampled so that cattle used in the study could be traced in future if there was a need to do so.

The blood was collected from the jugular vein of cattle that were selected. Blood was collected using blood sampling tubes (Figure 3.4). A total of 392 cattle were randomly selected from various crush pens in all constituencies of Ohangwena region.

Figure3.4: The researcher collecting blood from jugular vein of cattle using the red top vacutainer (7 ml) tubes

The following baseline parameters were recorded for each animal that was sampled: age, sex and crush pen were it was sampled from. It is a legal requirement in Namibia for all cattle from the age of two months to be identified with two ear tags (electronic on left ear and visual on right ear) with a unique number for each cattle. As mentioned above, blood was

only collected from cattle that had ear tags (Figure 3.3). Therefore, it was easy to trace the animals that were sampled during the course of the study and even after the study if there was a need.

Information about owners was accessed from the NamLits (Namibia Livestock Identification and Traceability System) database found at the state veterinary offices throughout the country.

3.1.5 Collection of blood samples

All blood samples were collected either in EDTA tubes (7ml) or red top vacutainer (7 ml) tubes, because the laboratory could detect antibodies either from coagulated blood or no coagulated blood (Figure 3.5).

Blood samples were collected from from the jugular vein of cattle restrained in the crush pen by a trained Animal Health Technician. The latter followed the instructions of the laboratory. The puncture sites from where blood was collected were treated with wound spray to prevent infection. The blood was let to clot at room temperature for a few hours or centrifuged to separate serum. The serum was then kept at -20 ^{o}C until it was sent for analysis at the Agricultural Research Centre, Onderstepoort Veterinary Institute, Pretoria.

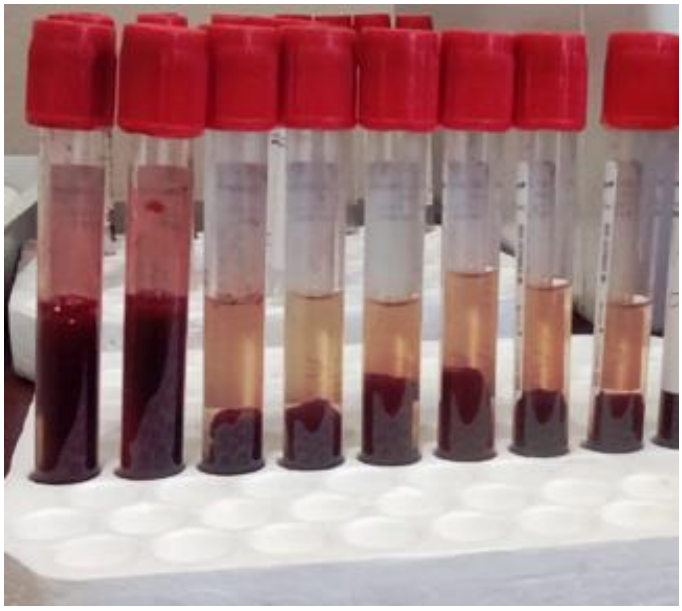

Figure3.5: Blood samples ready for centrifuging to separate serum and blood cells

3.1.6 Experimental procedures

Samples were centrifuged in Ondangwa State Veterinary Laboratory before they were packed and forwarded to the Agricultural Research Centre, Onderstepoort Veterinary Institute, Pretoria, where the serological test; Indirect Fluorescent Antibody (IFA) was used to detect antibodies against *Babesia bovis and Babesia bigemina*.

IFAT method was employed in this study to test for presence of antibodies against the causes of bovine babesiosis. The method was selected based on the OIE recommendation for epidemiological surveys and because it is a widely used method for detecting the antibodies of *Babesia bovis* and *Babesia bigemina* (OIE 2013)**.**

The uses of microscopic method, such as blood smear are recommended for clinical cases, but not suitable for epidemiological surveys. This is because it is limited by the low parasitaemia during the acute phase (generally observed at the beginning of parasite multiplication) but also the number of samples that can possibly be examined in a day (Vetter 2012; Gonçalves Ruiz et al. 2001).

3.1.7 Processing samples and test procedures

The anticoagulant (EDTA) tubes with sera were received at the laboratory. The sera were then washed three times in eight volumes of phosphate-buffered saline (PBS) to remove contaminating plasma proteins and, in particular, host immunoglobulins. After washing, the infected red blood cells (RBCs) were re-suspended in two volumes of PBS to which 1% bovine serum albumin (BSA) had been added to adhere RBCs to the glass slide. A single-layered blood film was made by placing a drop of blood on to a clean glass slide, which was then spun in a cytocentrifuge to produce uniformed smears. The films were air-dried and fixed for five minutes in an oven at 80°C. Fixed blood films were then covered with aluminium foil, rendered airtight, and stored at –70°C until required for test procedures.

Test sera were diluted 1/30 in PBS. The slides were marked into 8–10 divisions with an oil pen to produce hydrophobic divisions. In each test square, 5–10 µl of each serum dilution was added to a filter paper disc using a fine pipette. The preparations were then incubated at 37°C for thirty minutes, in a humid chamber. For controls, negative and weak positive sera (at the same 1/30 dilution) were used on each test slide.

After incubation, the slides were rinsed with a gentle stream of PBS to remove the filter paper discs. The slides were soaked for ten minutes in racks in PBS followed by ten minutes in water. The PBS and water were circulated using a magnetic stirrer. Diluted anti-bovine immunoglobulin G (IgG) antibody labelled with fluorescein isothiocyanate (FITC) was then added to each test square. The slides with the conjugate were incubated again at room temperature for thirty minutes, and washed as above. The wet slides were mounted with cover-slips in a solution containing one part glycerol and one part PBS, and examined by standard fluorescence microscopy.

Positive controls contained focal clusters of cells exhibiting apple green cytoplasmic Fluorescence, while negative controls did not exhibit fluorescence.

Suspect sample, which appeared similar to the positive control at any one of the four times, were considered positive. While samples without appropriate fluorescence at any of the four time periods were considered negative.

3.1.8 Data Processing and Statistical Analysis

Data was captured using Microsoft Excel. Before analysis commenced, the data was checked for any inconsistences and improbable entries. Data was then analysed using IMB SPSS statistics version 23. Descriptive statistics were presented as graphs and tables. EpiTools epidemiological calculators found on the site developed and maintained by Ausvet, was used to calculate the prevalence, and compute the confidence intervals for the prevalence estimates (Sergeant 2015). The Chi-square and Fisher's exact test was used to assess the association between the diseases status (positive=1 or negative=0) and categorical covariates like age, sex, and place.

Chapter 4

Results

4.1 Summary statistics

The following sections provide a detailed description of the descriptive statistics of the various variables.

4.1.1 Number of cattle sampled

Table 4.1, shows the number of samples collected from each constituency by age and sex of cattle. The majority (63.01%; 247/392) of cattle sampled were adult cattle (≥ 5 years old), followed by middle-aged cattle (3-4 years old) (22.96%; 90/392). Cattle < 2 years and below contributed the least (14.03%; 55/392) number of samples.

Table4.1: Profile of cattle sampled in Ohangwena region (n=392)

Constituencies	Number of cattle					
	Frequencies n (%)	Sex		Age groups		
		Female n (%)	Male n (%)	0-2 years n (%)	3-4 years n (%)	≥5 years n (%)
Okongo	136 (34.7)	69 (17.6)	67 (17.1)	23 (5.9)	38 (9.7)	75 (19.1)
Endola	40 (10.2)	20 (5.1)	20 (5.1)	4 (1.0)	10 (2.6)	26 (6.6)
Ongenga	32 (8.2)	15 (3.8)	17 (4.3)	2 (0.5)	11 (2.8)	19 (4.8)
Ondobe	32 (8.2)	20 (5.1)	12 (3.1)	5 (1.3)	6 (1.5)	21 (5.4)
Omulonga	32 (8.1)	13 (3.3)	19 (4.8)	5 (1.3)	6 (1.5)	21 (5.4)
Eenhana	24 (6.1)	13 (3.3)	11 (2.8)	4 (1.0)	2 (0.5)	18 (4.6)
Ohangwena	24 (6.1)	12 (3.1)	12 (3.1)	0 (0.0)	7 (1.8)	17 (4.3)
Omundaungilo	24 (6.1)	13 (3.3)	11 (2.8)	4 (1.0)	2 (0.5)	18 (4.6)
Epembe	24 (6.1)	14 (3.6)	10 (2.6)	4 (1.0)	5 (1.3)	15 (3.8)
Engela	16 (4.1)	6 (1.5)	10 (2.6)	1 (0.3)	3 (0.8)	12 (3.1)
Oshikango	8 (2.1)	5 (1.3)	3 (0.8)	3 (0.8)	0 (0.0)	5 (1.3)
Total	**392** (100)	**200** (51.0)	**192** (49.0)	**55** (14.0)	**90** (23.0)	**247** (63.0)

The largest number of samples were collected from Okongo constituency (34.7%; 136/392) followed by Endola that contributed 10.2% (40/392). Omundaungilo, Epembe, Eenhana and Ohangwena constituencies contributed 6.1% (24/392) each to the total number sampled. Each of Ondobe, Omulonga and Ongenga constituencies contributed 8.2% (32/392). Oshikango constituency contributed the least number of samples (2%; 8/392).

4.1.2 Spatial distribution of *Babesia* species in the study area.

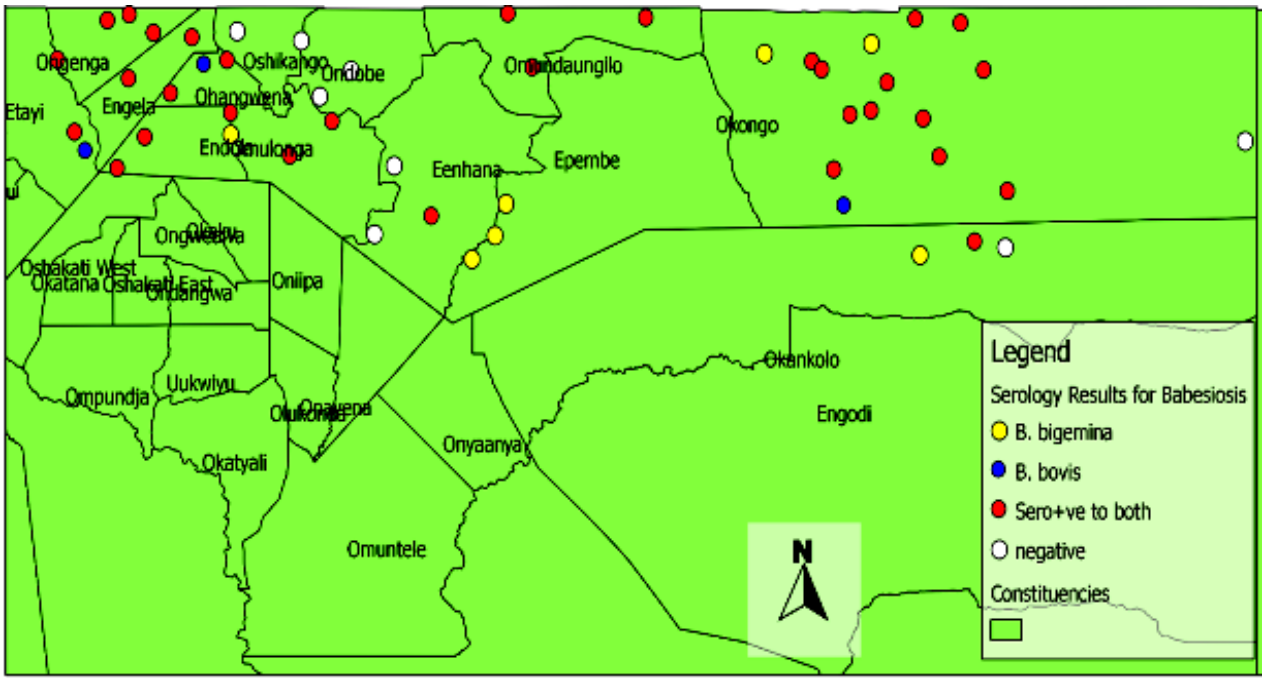

Figure4.1: Distribution of babesiosis at crush pens in Ohangwena region

Figure 4.1 shows the distribution of the *Babesia* species in Ohangwena region. The parasites were found throughout the region except in Oshikango constituency. Likewise, mixed infections were observed in all constituents with the exception of Ondobe and Oshikango constituency. Infections with only *Babesia bigemina* were detected at three foci in Epembe, and Okongo constituencies, and at one focus in Endola constituency. Infections with only *Babesia bovis* were detected at one focus in each of Okongo, Ohangwena and Engela. The map shows that, the parasites mostly concentrated in the eastern part of the region (Okongo constituency) and far western parts of the region in comparison to the central region.

Ongenga constituency had the highest prevalence of *Babesia bovis* (37.5%, 95% CI: 22.9-54.7), (Table 4.2).

Table4.2: Proportion of samples positive for *Babesia bovis* in constituencies (n=392)

Constituencies	Frequency (n)	Prevalence (%)	95% Confidence Interval	
			Lower	Upper
Okongo	26/136	19.1	13.3	26.2
Omundaungilo	2/24	8.3	2.3	25.8
Epembe	3/24	12.5	4.3	31
Eenhana	1/24	4.2	0.7	2.0
Omulonga	4/32	12.5	5	28.1
Ondobe	1/32	3.1	0.6	15.7
Ohangwena	2/24	8.3	2.3	25.8
Engela	2/16	12.5	3.5	36
Ongenga	12/32	37.5	22.9	54.7
Oshikango	0/8	0	0	3.2
Endola	12/40	33.3	19.1	47.5
Estimated prevalence	**65/392**	**16.6%**	**13.2**	**20.6**

Likewise Ongenga yielded the highest number of *Babesia bigemina* (65.6%, 95% CI: 48.3-79.6) (Table 4.3). There was no positive cases in Oshikango constituency (0/8; 0 %, 95% CI: 0-3.2) for all species.

Table4.3: Proportion of samples positive for *Babesia bigemina* in constituencies (n=392)

Constituencies	Frequency (n)	Prevalence (%)	95% Confidence Interval	
			Lower	Upper
Okongo	50/136	36.8	28.7	44.5
Omundaungilo	11/24	45.8	27.9	64.9
Epembe	5/24	20.8	9.2	40.5
Eenhana	3/24	12.5	4.3	31
Omulonga	10/32	31.3	18	48.6
Ondobe	4/32	12.5	2.3	25.8
Ohangwena	14/24	58.3	38.8	75.5
Engela	4/16	25	10.2	49.5
Ongenga	21/32	65.6	48.3	79.6
Oshikango	0/8	0	0	3.2
Endola	21/40	52.5	39.7	69.9
Estimated prevalence	**143/392**	**36.5%**	**31.9**	**41.4**

Endola constituency yielded the highest number of mixed infections (12/40; 30.0%, 95% CI: 19.1-47.5). The lowest prevalence was found in Oshikango constituency with 0/8 (0 %, 95% CI: 0-3.2) for all species (Table 4.4)

Table4.4: Proportion of samples positive for mixed infections in constituencies (n=392)

Constituencies	Frequency (n)	Prevalence (%)	95% Confidence Interval	
			Lower	Upper
Okongo	19/136	13.9	9	20.5
Omundaungilo	2/24	8.3	2.3	25.8
Epembe	2/24	8.3	2.3	25.8
Eenhana	1/24	4.2	0.7	20.2
Omulonga	1/32	3.1	0.6	15.7
Ondobe	0/32	0	0	10.7
Ohangwena	2/24	8.3	2.3	25.8
Engela	2/16	12.5	3.5	36
Ongenga	10/32	31.3	18	48.6
Oshikango	0/8	0	0	3.2
Endola	12/40	30.0	19.1	47.5
Estimated prevalence	**51/392**	**13%**	**10**	**16.7**

4.1.3 The overall prevalence of babesiosis in the study area

Out of the 392 specimens that were tested, 16.6 % (95% CI: 13.2-20.6) were positive for *Babesia bovis*, 36.5 % (95% CI: 31.9-41.4) were positive for *Babesia bigemina*, and 13% (95% CI: 10-16.7) were positive for mixed infections (Table: 4.5).

Table4.5: Frequencies and prevalence of positive samples

***Babesia* species**	Frequencies of positive samples (n)	True prevalence (%)	95% Confidence Interval	
			Lower	Upper
Babesia bigemina	143	36.5%	31.9	41.4
Babesia bovis	65	16.6%	13.2	20.6
mixed infections	51	13%	10	16.7

Overall, *Babesia bigemina* was more prevalent with (36.5 %) as compared to *Babesia bovis* (16.6%), while mixed infections were the least (13%). Using the proportions of positive samples obtained, the true prevalence of the disease was then estimated using the EpiTools epidemiological calculators (Sergeant 2015). *Babesia bigemina* had the highest true

prevalence at 36.5% (143/392). This was followed by *Babesia bovis* at (16.6% (65/392) and the mixed infections at 13% (51/392) (Figure 4.2).

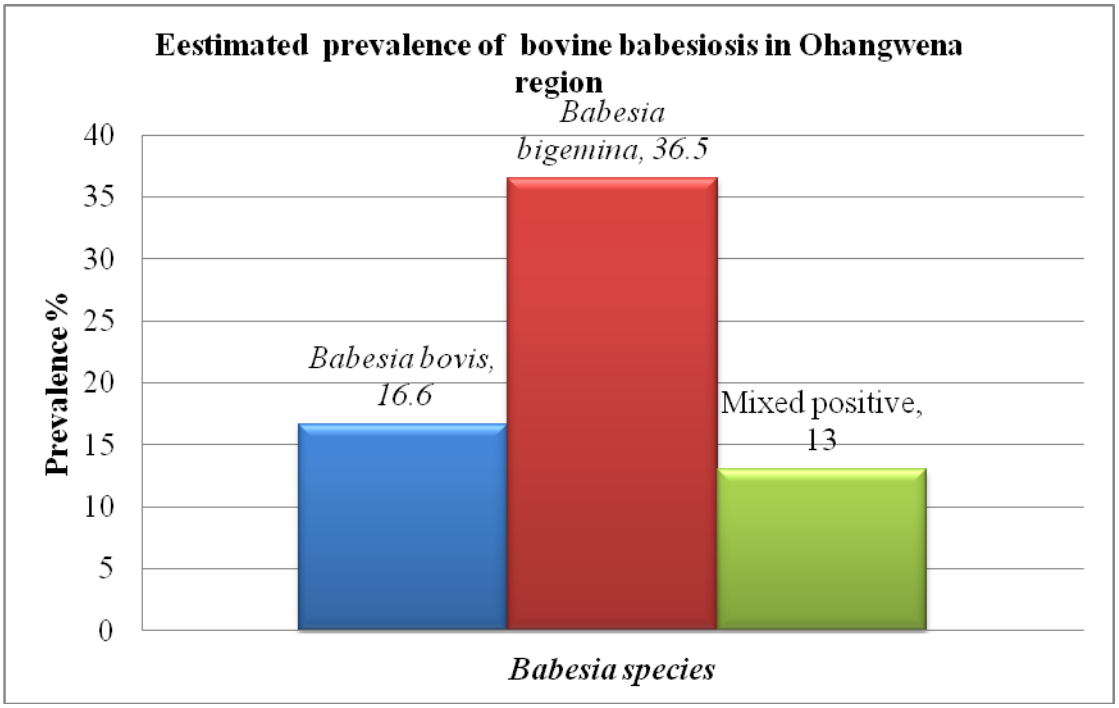

Figure4.2: The prevalence of *Bovine babesiosis* in Ohangwena region

Assessment of the prevalence of infection in the individual constituencies (Figure 4.3) showed that Ongenga constituency had the highest prevalence of both *Babesia bigemina* (65.6%, 95% CI: 48.3-79.6) and *Babesia bovis* (37.5%, 95% CI: 22.9-54.7). Endola constituency had the highest prevalence of mixed infections (30.0%, 95% CI: 19.1-47.5). The lowest prevalence of *Babesia* parasites was found in Oshikango constituency that recorded no infection (0 %, 95% CI: 0-3.2).

In all the constituencies, *Babesia bigemina* had the highest prevalence. Only one constituency; Oshikango did not have a positive case (Figure 4.3).

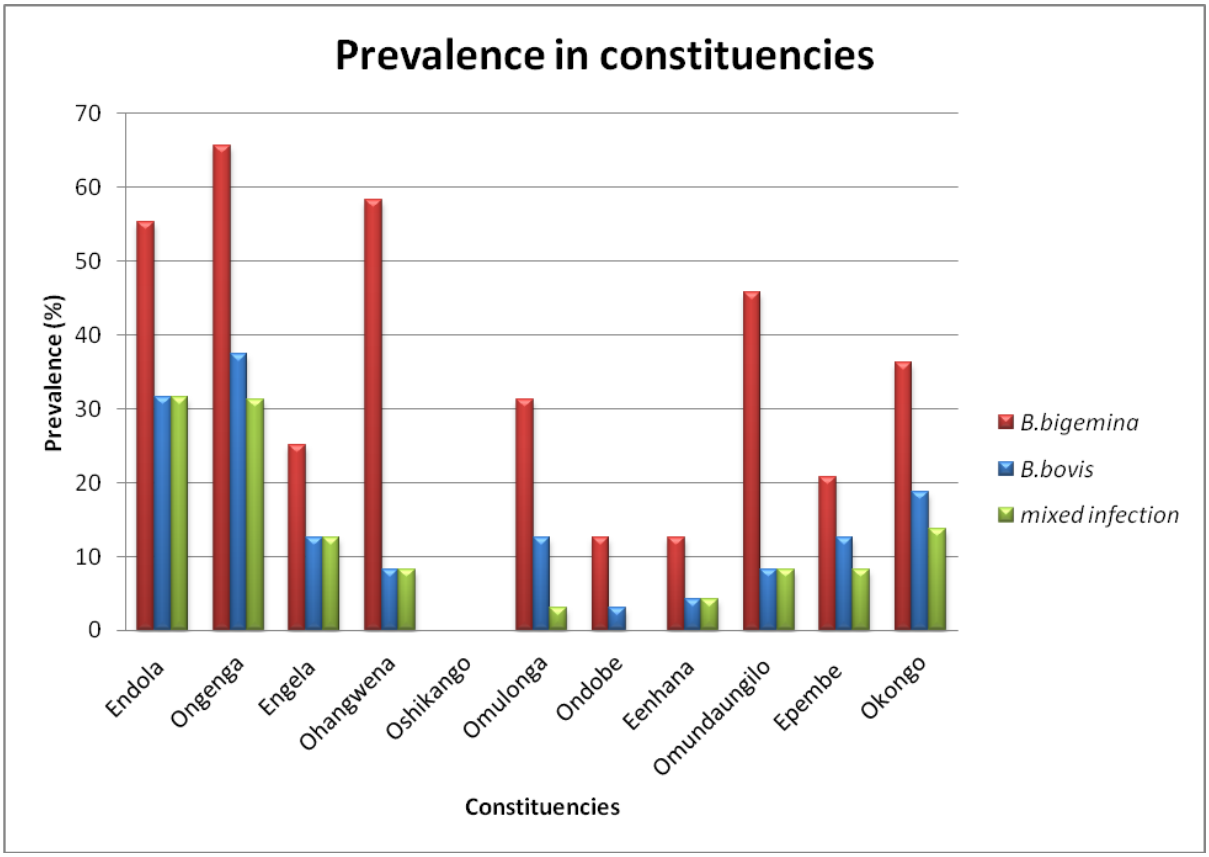

Figure4.3: Prevalence of babesiosis in the various constituencies of Ohangwena region

4.1.4 Prevalence of babesiosis based on sex and age of cattle in Ohangwena region

Babesia bigemina was the most common species in both males (38.5%, 95% CI: 31.8-45.3) and in females (34.5%, 95% CI: 28.0-40.6). On the other hand, *Babesia bovis* had a prevalence of 14.6% (95% CI: 9.9-19.8) in males and (18.5%, 95% CI: 12.9-24.5) in females.

Mixed infections were observed in only 10.9% (95% CI: 6.8-15.6) of the males and 15% (95% CI 9.4-20.5) of the females (Table 4.6). The most common species in all age groups was *Babesia bigemina* with 44.6% (95% CI: 34.8-55.6) in middle age group (3-4 years) and 35.0% (95% CI: 29.2-40.8) in adult cattle (5 years and above). *Babesia bovis* had the next highest prevalence (23.9%; 95% CI: 15.0-32.8) in the middle age group, followed by age group of adult cattle at 14.8% (95% CI: 10.3-18.9) prevalence. Mixed infections had the least prevalence (18.5%, 95% CI: 9.8-26.3), and this was in the middle-aged cattle (Table 4.6).

Table 4 6: Proportion of positive samples based on sex and age of cattle in the Ohangwena region (n=392)

	SEX				**AGE GROUP (YEARS)**					
	Male (n=192)		**Female (n=200)**		**0-2**		**3-4**		**≥5**	
***Babesia* Spp.**	**n**	**%(CI)**	**n**	**% (CI)**	**N**	**% (CI)**	**n**	**% (CI)**	**n**	**%(CI)**
Babesia bovis	28/192	14.6 (9.9-19.8)	37/200	18.5 (12.9-24.5)	7/55	12.3 (3.5-22.8)	22/90	23.9 (15.0-32.8)	36/247	14.8 (10.3-18.9)
Babesia bigemina	74/192	38.5 (31.8-45.3)	69/200	34.5 (28.0-40.6)	17/55	29.8 (17.6-42.1)	41/90	44.6 (34.8-55.6)	85/247	35.0 (29.2-40.8)
Mixed infections	21/192	10.9 (6.8-15.6)	30/200	15(9.4-20.5)	5/55	8.8 (1.8-17.5)	17/90	18.5 (9.8-26.3)	29/247	11.9 (7.4-15.6)

4.2 Inferential statistics

In the section that follows, results of the assessment of association between the dependent and independent variables are reported. The dependent variable is infection status, while the independent variables are age, sex and place.

4.2.1 Association between place and infection

The association between the number of infected animals and the place was not statistically significant (P>0.05)

Babesia bovis (Chi square = 66, Degrees of freedom 60, p-value = 0.28), *Babesia bigemina* (Chi square = 108, degree of freedom = 99, p-value = 0.25) and for mixed infections (Chi square = 72, degree of freedom = 66 p-value = 0.29). The test for association between place and infection was not statistically significant for all species (P>0.05).

4.2.2 Association between age of the animal and infection

The relationship between the age of animals and infection was tested for each individual species (*Babesia bovis* and *Babesia bigemina*), and the relationship between the two was not statistically significant (P>0.05) (Table 4.7 and 4.8).

Table4.7: The association between the age of cattle and infection of *Babesia* infection

Positive for *Babesia*	**Age Category**			
	0-2 years	**3-4 years**	**≥ 5 years**	**Total**
Positive	19 (33.3%)	46 (50%)	92 (37.9%)	157
Negative	38 (66.7%)	46 (50%)	151(62.1%)	235
Total	**57**	**92**	**243**	**392**

Chi -square = 12, degree of freedom = 9, P-value = 0.21

Table4.8: The association between age of cattle and infection by *Babesia* species

Infected with *Babesia bovis*	**Age Category**			
	0-2 years	**3-4 years**	**≥5 years**	**Total**
Positive	7 (12.3%)	22 (23.9)	36 (14.8)	65
Negative	50 (87.7)	70 (76.1%)	207 (85.2)	327
Total	**57**	**92**	**243**	**392**
chi -square = 12, degree of freedom = 9, P-value = 0.21				
Babesia bigemina	**0-2 years**	**3-4 years**	**≥5 years**	**Total**
Positive	17(29.8%)	41 (44.6%)	85 (35%)	143
Negative	40 (70.2%)	51 (55.4%)	158 (65%)	249
Total	**57**	**92**	**243**	**392**
chi -square = 12, degree of freedom ==9, P-value = 0.21				
Mixed infection	**0-2 years**	**3-4 years**	**≥5 years**	**Total**
Positive	5 (8.7%)	17 (18.5%)	29 (11.9%)	51
Negative	52 (91.3%)	75 (81.5%)	214 (88.1%)	341
Total	57	92	243	392
chi -square = 12, degree of freedom = 9, P-value = 0.21				

4.2.3 Association between sex of cattle and infection

Like was observed for age, the association between the number of infected animals and sex of the animal was not significant (P>0.05), (Table 4.9).

Table4.9: Association between sex of cattle and infection

Sex/ gender	Positive	Negative	Total
Female	76	124	200
Male	81	111	192
Total	**157**	**235**	**392**

Chi^2= 12, degree of freedom =9, P value =0.21

Chapter 5

Discussion

5.1 Distribution of cattle in the study area

The practice of allowing older cattle to live beyond a productive period, influences the occurrences of the disease by increasing the population of carrier animals (Tschopp et al. 2010). As a result, keeping old animals has epidemiological importance for babesiosis. It has been reported that older cattle become carriers of bovine babesiosis. Therefore when a herd has more older animals, it directly promotes and enhances the spread of bovine babesiosis (Góes et al. 2008; Alaa et al. 2011; Cárdenas-Canales et al. 2011).

5.2 Spatial distribution of *Babesia* species in the study area

The present study was conducted on local sanga cattle to evaluate the distribution of bovine babesiosis in Ohangwena region. The findings of the study revealed the presence of antibodies against *Babesia* species in cattle. This indicates the presence and exposure to babesiosis in the last 12 months in the study area. This was expected given that; farmers report cases of sick animals to state veterinary offices. These also include cases of babesiosis. In addition there have been confirmed cases of babesiosis in the study area (Republic of Namibia 2013). Furthermore, the findings of the present study were consistent with the existing literatures where Namibia and other countries of southern Africa were found to be endemic to bovine babesiosis (Pfitzer 2009; Marufu 2008; Canever et al. 2014).

Results of this study show that the disease was widely distributed throughout the study area. *Babesia bovis* as a single infection was found to be less spatially distributed as compared to *Babesia bigemina* (Figure 4.1). However, *Babesia bigemina* had a high distribution at the crush pens in the central part of the region bordering Eenhana and Epembe constituencies, and towards the eastern part of the region in Okongo constituency (Figure 4.1). This might be associated with the distribution of vector ticks in the area, and could explain why the distribution of *Babesia* infections reduced from the eastern part of the study area towards the central part of the region, and again increased in the eastern part of the study area.

The distribution of bovine babesiosis in the study area was not uniform. Some areas had high frequencies than others (Figure 4.1). The differences observed may have resulted from differences in the micro-climate in the region. Constituencies to the western part of the study area (Engela, Endola, Ongenga and Ohangwena) were more prone to floods than the eastern part of the study area. Therefore, given the wet conditions in the western parts of the study area, the environment was likely to be more conducive for growth and multiplication of the vector ticks. According to Coskun (2012), wet environments tend to be favourable for breeding and growth of ticks. This could lead to an increase in the tick population in the west as compared to the other areas to the east of the study area. This could be the reason why high frequencies of *Babesia* were observed in the western part of the study area as compared to the eastern part of Ohangwena region.

In the sub-Saharan Africa, *Babesia bigemina* possess wider distribution, following the distribution of its main vector ticks *R. (Boophilus) decoloratus* and *R. evertsi* (Latif & Walker 2004; Robbins 2012). The difference in the distribution of the two species, could be due to the fact that *Babesia bigemina* has five various possible vector ticks while there are only two vector ticks for *Babesia bovis* (Hesterberg 2007; Baumgärtner & Tikubet 2012).

The spatial variation in the distribution of *Babesia* parasites concurred with studies conducted in other African countries and beyond (Muhanguzi et al. 2014; Yeruham et al. 1998; Irwin 2009).

Although it was not the objective of this study to assess the availability of drugs, it was observed that, some farmers in villages had limited access to pharmacies where they could source acaricides to treat their cattle for ticks. Such a situation hampers the farmer's ability to procure remedies. This could hence have contributed to the poor rate of tick control in the grazing area (Republic of Namibia 2015), and also explain the difference in the distribution of the disease that was observed in this study.

Lack of a uniform tick control strategy in the area could also have played a role in the variation in the spatial distribution that was observed in the study area. The government only provides annual vaccination for lung sickness (Contagious Bovine Pleuropneumonia) to cattle (Republic of Namibia 2015). Tick control remains the responsibility of individual farmers (Republic of Namibia 2011). The use of acaricides has been the most widely employed

system to control ticks on cattle worldwide. However, the improper use of chemicals in communal farming areas can lead to the development of tick resistance to various acaricides (Mekonnen et al. 2002; Serra-Bonveh & Orantes-Bermejo 2010). In addition to resistance, farmers need to be cautioned to avoid the problem of chemical residues in meat, milk and in the environment. In view of this, the Directorate of Veterinary Services of Namibia should come up with a tick control strategy that is effective and efficient. In addition, government may also have to implement safety measures related to the use of chemicals for treatment of animals.

Technical know-how has been identified as one of the factors, affecting the efficacy and effectiveness of an acaricides (Hangara et al. 2011). In communal areas, use of acaricides at the incorrect concentration has resulted in the tick control failure in some counties (OIE 2013; Mekonnen et al. 2002). In order to avoid all these problems, veterinary services need to ensure proper handling and use of these chemicals. Financial implications in addition to acaricides failure might in case of breakdown of dipping efficacy be considered as a contributing factor to the occurrence of the disease in the study area. Moreover, available literature proves that, chemical control could be unsuccessful because of its tendency to partially remove one parasite while causing the invasion of others which were not affected. This has been reported in south eastern African countries where the eradication of *R. decoloratus* ended up with the invasion of *R. microplus* (Yokoyama et al. 2006; Suarez & Noh 2011; Olwoch et al. 2008).

In addition, it is possible that the animal health management in terms of tick control was poorly executed by farmers in the study area. This may be the reason that cattle in the study area got infected by *Babesia* species. The technical know-how on handling and application of acaricides might also be a challenge to communal farmers as most of them are not able to read and write especially in English. There are several chemicals that are well-known and are highly recommended for control of ticks using either the spray race, hand dressing and other methods (George et al. 2004; Reginald De Deken, Ivan Horak, Maxime Madder 2014). However, if the control strategies are not applied in the correct manner, it can also contribute to increased prevalence of the disease in the area.

Apart from a wide distribution of the disease, the indigenous *Bos indicus* cattle in the area could be crossed with large framed breed with weaker resistance to tick borne diseases. This could lead to production of good meat producers and heamoparasites resistant cattle. This method has been successfully employed in other counties such as Australia (Olivia 2015; George et al. 2004).

Risk mapping might also be necessary to complement existing control methods. Understanding and mapping the movement of cattle from villages to the cattle posts as they change in space and time may help to understand the epidemiology of bovine babesiosis in the area. Cattle could be dipped before moving from the west of the region to the areas where the disease has lower distribution. This might be a prerequisite to sustainable control and reduce the distribution of tick vectors in the study area.

The common phenomenon of moving and displacing cattle in search of better grazing areas, plays a significant role in disease distribution. This causes changes in the host population and/or parasite diversity. The absence of host may disrupt the life cycle of the ticks in the study area. Such changes affect the timing of tick-borne infections and thus place a burden on the nation's tick-control programmes (Olwoch et al. 2008; Penzhorn 2015; Tembue et al. 2011) .

Tick fever presents a serious barrier on international cattle trading (Bock et al. 2004; OIE 2013). As in many other countries, tick fever continues to pose threats to cattle farming in Namibia. As Namibia strives to provide beef and dairy produce to other countries, the control of tick borne diseases needs to be emphasised (Republic of Namibia 2013).

In addition, indirect costs of babesiosis in endemic areas remained underestimated. Generally, animals from tick free areas tend to be highly susceptible to tick borne diseases. Cattle suffer acute form of the disease and many die in the weeks following their arrival in the disease endemic areas. The consequence is that, the quality of cattle in endemic areas remains low, therefore impeding the development of the cattle industry and the well-being of farmers (Gualito et al. 2012).

According to Schnittger et al. (2012) and Lew & Jorgensen (2005), calculation of the overall impact of ticks and tick-borne diseases in different countries showed that the cost incurred in loss and control of babesiosis and anaplasmosis alone cost the Australian cattle industry US$16.9m per annum with 'tick worry' adding US$6.4m to annual losses. The cost in eastern and southern African counties (Zimbabwe, Tanzania, and South Africa) was estimated in the range of 5.1 to 21.6 million US dollars annually. We do not know the cost of tick-borne diseases in the study area.

5.3 The prevalence of babesiosis in the study area

Babesia parasites are mostly found in pastures where vector ticks prevail, especially in countries in the tropical and subtropical zones (Atif et al. 2013). As shown in Figure 4.2, the true prevalence in the current study was estimated at 16.6 % (95%; CI: 13.2-20.6) for *Babesia bovis* and 36.5 % (95%; CI: 31.9-41.4) for *Babesia bigemina*. These results correspond to a great extent with outcomes of a surveys conducted in some areas of various countries around the globe. For example, in Mozambique, the prevalence of *Babesia bovis* and *Babesia bigemina* were 76.0% (528/695) and 78.8% (548/695) respectively (Tembue et al. 2011). The Mozambique study reported a higher prevalence as compared to the current study.

When studying the prevalence of bovine babesiosis, the influence of natural immunity should be considered. The presence of *Babesia* antigen in the form of living parasites inside the vertebrate host is considered to be the main factor for good immunity (Rahman, Lye & Chandrawathani 2010; Hedimbi et al. 2011). If cattle have been infected by parasites; may continue to have detectable antibodies for many years after the infection. This may even continue for some time after the parasite has been eliminated from the environment (Alaa et al. 2011).

The IFA used in the current study is able to detected antibodies in animals that have been infected within 6-12 months. After six to twelve months of the infection, the antibodies for bovine babesiosis diminish, and the animal becomes immune, and hence tests negative (OIE 2012b). The current study detected antibodies in calves above one year of age, of which the maternal immunity should have diminished. This indicated that there was continuous infection of cattle, and the presence of parasitic infected vector ticks in the environment.

This information is useful as it can help to define the epizootiological balance in the population and to determine control strategies against the disease (Rahman, Lye, Chandrawathani, et al. 2010)**.**

On the other hand, findings of the present study showed a relatively high prevalence as compared to the findings from the Bie province of Angola where the overall prevalence of babesiosis was relatively low, with the prevalence of *Babesia bigemina* at 1% and *Babesia bovis* at zero (Kubelová et al. 2012). However, findings of the present study concurred with some surveys conducted in some provinces of South Africa that also reported the presence of *Babesia* antibodies in cattle. For example, Mtshali et al. (2013) conducted a serological prevalence study on multiple tick borne pathogens of cattle in the Free State province of South Africa. The prevalence was 1.4 % for *Babesia bovis* and 2.7% for *Babesia bigemina* respectively.

The prevalence of bovine babesiosis in the current study was higher than that observed in the neighbouring countries (Angola and South Africa). This was not expected given that Namibia is a semi-arid area (dryer than Angola and South Africa) and hence not conducive for survival of ticks that spread the disease (Republic of Namibia 2013). In view of this, the prevalence of babesiosis in Ohangwena region could be considered to be high. These findings also confirm that Ohangwena region was epidemiologically infected with bovine babesiosis with a risk of spreading to other areas such Angola. Hence veterinary services in Ohangwena region should focus a lot on reducing the incidence of babesiosis.

Another issue worth noting from this study was the detection of *Babesia bovis* in the region adjacent to the border with Angola. This finding showed that Ohangwena region posed the risk of spreading *Babesia bovis* into Angola or vice versa in event of infected cattle moving across the border.

In Namibia, there is a lack of current data on the prevalence of bovine babesiosis and other tick borne diseases. Isolated cases have been reported at various state veterinary offices in the country (Republic of Namibia 2013). Furthermore, although similar studies have been undertaken in other countries of southern Africa in recent years (Lew & Jorgensen 2005; Hesterberg 2007; Horak, et al 2012), up to the time of the current study, no study had been

conducted on or reported the prevalence of bovine babesiosis in the study area. Therefore through the current study, that uncertainty has been removed by demonstrating the sero-prevalence.

Various tick species such as *Rhipicephalus* Spp. etc. are the most likely known source for *Babesia* infections in the tropics (OIE 2013; Wormser et al. 2006). During collection of blood samples, the existence of ticks on the environment and infestation on cattle was observed. This observation validated the results of the current surveillance.

Findings of the study confirmed that *Babesia* parasites were circulating in the Ohangwena region. This is consistent with reports that have concluded that tick-borne diseases, especially theileriosis, babesiosis, and anaplasmosis, are prevalent in Africa and Asian countries (Rahman, Lye & Chandrawathani 2010). Since it was outside the scope of this study to establish the presence of other tick borne diseases in the study area, there is a need to determine the prevalence of other tick borne diseases in the study area. This would be of interest particularly to the government to decide if heamoparasites should be state controlled diseases or not, as part of the effort to improve cattle farming in the study area.

Like in other communal farming system of Africa, *Bos indicus* cattle (sanga cattle) of the southern African origin were the dominant breeds in the study area. This breed is considered to be naturally resistant to tick parasitism and tick-borne diseases (Jonsson et al. 2014). This could explain why the prevalence in this region for *Babesia bovis* (16.6 %) and *Babesia bigemina* (36.5%) was generally lower as compared to what other studies in areas where several breed types including the *Bos taurus* are reared. All cattle sampled in the study area, were indigenous sanga breed. The African Zebu and other African local breeds are reportedly known to be relatively resistant to ticks and tick-borne diseases as compared to exotic cattle breeds. Therefore the breed under study has a lower likelihood of developing clinical disease, when exposed to infection (Abdullah-Al-Mahmud M.D., Shariful Hoque Belal S.M. 2015). This reduces the risk of farmers losing their animals to the tick-borne diseases through rearing animals adapted to the conditions and known prevailing diseases in the area.

For ethical reasons, animals that were sampled were examined for any clinical symptoms of babesiosis during the collection of blood samples. It was rare to find animals displaying signs of babesiosis infection. This was probably because the breed in study was known to be

resistant to tick borne diseases or that animals might have been treated with Imidocarb diproprionate (1.2 mg/kg body weight) by their owners before the study.

Enzootic stability is an epidemiological concept where the rate of transmission is sufficient to infect the majority of calves before calf hood resistance is lost. In a situation of enzootic stability for babesiosis, calves should get infected during the early months of the first year of life, while protected by colostrum antibodies (passive immunity). Continued infection encountered after the diminishing of maternal immunity, enables the development of active immunity without the showing of a clinical disease (Hasle et al. 2010; De Vos & Potgieter 1983).

According to Horak et al. (2012) prevalence rates of babesiosis equal to or greater than 75.0% qualify the area to be considered as exhibiting enzootic stability. In the current study the detected sero-prevalence was lower than 75%, which means that the Ohangwena region of Namibia has not reached the level of enzootic stability for bovine babesiosis. In cases where the area does not attain endemic stability, the natural immunity is not sufficient in the herds. Therefore acaricides and vaccination should be used simultaneously to control the disease unlike in endemic stability situation, where chemicals are no more (Kubelová et al. 2012; Regitano & Prayaga 2010). This could explain the overall high prevalence (36.5 and 16.6%) of the disease observed in this study even though most of the animals looked healthy. The Namibian government should consider chemical control measures to combat bovine babesiosis in Ohangwena region.

In Ohangwena region, indigenous cattle are reared under traditional grazing systems that involves crop-livestock production and as a result, they reportedly get sufficient exposure to constant tick challenge (Tschopp et al. 2010; Madder et al. 2014). Therefore, the development of endemic stability to heamoparasites might occur with minimal suffering to diseases. Furthermore, some authors have suggested that the endemic stability is more likely to develop for *Babesia bigemina* than to *Babesia bovis* in areas where both species are present (Estrada-Peña & Salman 2013; Penzhorn 2015).

The results of the current study revealed that, the immunity of cattle in the study area was not boosted on time after maternal antibodies diminished. This situation could lead to the

emergence of clinical babesiosis in adult cattle in the study area. Cattle acquire passive resistance from colostrum that last for about two months. This will be followed by innate immunity to babesiosis from three to nine month of age (Zintl et al. 2005; Goff et al. 2002; Lemma et al. 2015). Therefore calves exposed to babesiosis during the first 6 to 9 months may not suffer clinical babesiosis and have the ability to develop a solid long-lasting immunity (De Vos & Bock 2000; Penzhorn 2015). But in situation of endemic instability, animals that fail to become infected during the first year of life may therefore develop severe, life threatening disease if they get exposed later in life, depending on the breed (OIE 2012b).

The congregation of cattle at communal water points and common grazing areas leads to high density of animals in such areas. This may explain the level of prevalence observed in this study. This is supported by findings of other studies, where climate was considered to have influenced the intensity of infestation of *R. microplus* (Estrada-Peña & Salman 2013)**.**

If the environment is not conducive to ticks, it partially reduces the tick population (Kearney et al. 2013). This could also explain why the level of prevalence of *Babesia* Spp. infection observed in the constituencies on the eastern part study area was lower than the prevalence on the west that experiences floods from time to time.

Knowing the prevalence of agents in each epidemiological unit in the country, the directorate of veterinary services can develop geo-referenced maps of regions of stability and instability to provide better control of enzootic disease in the country.

In conclusion, Ohangwena region does not exhibit enzootic stability for babesiosis, and there are risk factors in the area that favour transmission of babesiosis to susceptible animals coming into the study areas especially the breeds of *Bos taurus* with poor resistance to tick borne diseases. This has potential to hinder the development of the cattle industry and improvement of the cattle breeds in the study area.

5.3.1 Most prevalent *Babesia* species

The current study determines the most prevalent *Babesia* specie in sanga cattle in the study area. The results revealed that *Babesia bigemina* was the most prevalent species with overall prevalence of 36.5% as compared to *Babesia bovis* with only16.6%. *Babesia bigemina* dominated the prevalence in all aspects (spatial and host factors).

The results of the current study supported findings from previous studies that discovered that both *Babesia* species affecting cattle occur in the same place and that where both exist, the prevalence of *Babesia bigemina* infections tends to dominate the prevalence of *Babesia bovis* infection (OIE 2013; Torr et al. 2002).

The results of the current study are also consistent with the findings of other studies from neighbouring countries that observed that *Babesia bigemina* exhibited a higher prevalence than that of *Babesia bovis*. The fact that the prevalence of *Babesia bigemina* superseded the prevalence of *Babesia bovis* suggests that, the two species are independent of each other and hence the likelihood of cross reactivity is low (OIE 2013). This is similar to the findings of the study by Hesterberg (2007) in Kwazulu Natal province of South Africa that reported the prevalence level of *Babesia bigemina* to be below 20% while *Babesia bovis* was as high as 90%.

Contrary to the findings of the current study, other studies found the prevalence of *Babesia bigemina* similar to that of *Babesia bovis*. For example, Rahman et al. (2010) in a study conducted in Malaysia reported the prevalence of *Babesia bovis* (17%) and *Babesia bigemina* (16%) to be similar.

In terms of pathogenicity, *Babesia bovis* is generally more pathogenic than *Babesia bigemina* (Schnittger et al. 2012; R Bock 2006) . Researches in other countries have indicated that, cattle infected with *Babesia bigemina* became carriers for a few months, while cattle infected with *Babesia bovis* remain carriers of the disease for a long period (Lemma et al. 2015).

For purposes of this study, if an animal was infected with only one of the two species, this was termed as single infection. In this study, some cattle were found infected by either *Babesia bovis* or *Babesia bigemina*. Mixed infections occurred where cattle were infected with *Babesia bovis* and *Babesia bigemina* simultaneously. This was in agreement with a similar study done in Malaysia by Chanddrawathani & (2010), where cattle were found to be infected with either one of the *Babesia* specie (single infection), while some were infected with both *Babesia* parasites simultaneously (mixed infections).

5.3.2 Prevalence based on place

As it was discussed earlier in topics prior to this, the prevalence of babesiosis was found to be generally high in the western constituencies as compared to those in the east. The highest prevalence was observed in Ongenga constituency at the rate of 65.6%, (95% CI: 48.3-79.6), and the species involved was *Babesia bigemina.*

At constituency level, babesiosis was present in all the constituencies. However, high prevalence rates were found in constituencies to the west of the region (Endola, Ongenga and Engela), with the lowest concentration in the central region. The slight rise of prevalence in Okongo might be resulted from continuous migration of cattle from the western part of the region in search for better grazing at cattle posts into Okongo constituency. As mentioned earlier, this was expected because, the large part of the grazing area of the western region consisted of flood water pans.

Surprisingly, the highest population of cattle with co-infections was found in Endola constituency with the prevalence level of 30.0%, (95% CI: 19.1-47.5). This was not anticipated since the geographic nature of Ongenga and Endola constituencies was similar.

Based on the true prevalence, analysis at constituency level for both *Babesia* species appeared in all constituencies except for Oshikango constituency. The prevalence recorded in Oshikango constituency (0%), may the resulted from the small grazing space in the constituency. More space in the constituency was occupied by the towns and high human population. Generally the constituency was smaller in size as compared to other constituencies (Figure 4.1).

Flooding is a recognised trigger to outbreaks of tick-borne diseases, in particular to bovine babesiosis. The accumulation of surface water in the pasture land, favour the breading of ticks. This leads to increased tick population and eventually abundant tick infestation on animals (Ursula J. Blumenthal, Jay M. Fleisher 2001; de Castro et al. 1997; Holechek 2013). This could explain why the prevalence of the disease seemed to be more concentrated in the western areas as compared to the central and eastern part of the study area. It might also be possible that, the variation of prevalence observed between the east and west might be due to the seasonal increase in the tick population especially during rainy season.

These data demonstrated that, the disease has a varied prevalence within the study area. The rate and variation of tick control practices by farmers may have influenced this difference. Therefore veterinary services need to put more efforts in emphasising proper and regular control of ticks. Regular surveillance of the prevalence of heamoparasites should be carried out in order to confirm the variations in prevalence of parasites as observed in the current study.

It is also possible that the differences observed may be due to a variation in the rate of tick infestation that subsequently affected a variation in the rate of cattle inoculated by the infected ticks. In addition, it is also possible that the risk of exposure of cattle to *Babesia* infection was also different. Cattle on the western part of the region were forced to graze in open water pans, due to the nature of the grazing area. These animals were more at risk of infections as compared to cattle residing in the eastern bushy part of the region.

The chi square analysis of the association of infection in constituencies was not statistically significant ($P>0.05$; chi= 0.28 *Babesia bovis* and 0.25 *Babesia bigemina*). This means that in the event of controlling bovine babesiosis in Ohangwena region, the entire areas should be considered as having a similar level of risk of acquiring infection.

The finding of the current study can serve as a tool for the development and implementation of future interventions and control strategies in animal health to fight tick borne diseases. So, for the control and eradication of bovine babesiosis, in Ohangwena region, epidemiological surveillance is an important aspect along with more attention to the reduction of tick population and reproduction. Government intervention through awareness programmes should be undertaken with the involvement of stakeholders in the livestock industry as well as consumers to avert risk to public health and economic losses associated with tick borne diseases in general.

5.3.3 Prevalence based on sex and age

In the current study a slightly higher prevalence rate in male cattle (42%) was recorded, as compared to female cattle (38%). However, the difference in the prevalence of sero-positivity between the two sexes of the cattle as not statistically significant ($P>0.05$). This is consistent with findings of previous studies that observed similar findings (Tembue et al. 2011).

The reason, why in the current study it was observed that both sexes were found to be equally exposed to *Babesia* infection, is the fact that cattle usually graze together in the veld and overnight in paddocks. This practice is likely to facilitate the transfer of ticks within the herds and eventually, facilitated the equal distribution of ticks among cattle leading to equal exposure in both sexes (Abdullah-Al-Mahmud M.D., Shariful Hoque Belal S.M. 2015).

Based on the findings of the present study, both sexes of cattle was equally susceptibility to *Babesia* infection. This is in agreement with the report by Lemma et al. (2015) who found equal prevalence in female and male cattle, with no statistically significance differences ($P>0.05$). Therefore, sex did not influence the prevalence of babesiosis in the current study.

The age of the cattle is one of the most important factors that influence the occurrence of bovine babesiosis. Literally, the occurrence of tick fever increases as the proportion of the age of the animal increases (OIE 2012a; Zintl et al. 2005). In the current study , the highest prevalence of babesiosis was found among the age group of 3-4 year old (50 %), followed by age group of cattle above 5 years old (37.9 .%). Calves aged between 0-2 years old had the lowest prevalence (33.3%).

However, the association between the age groups and infection observed was not to be statistically significant ($P > 0.05$). These findings are similar to those reported by (Vannier & Krause (2009); Vos & Waal (2006) and Bock et al. (2006) , who reported that, the infection of babesiosis increase with age of cattle. This could explain why calves appeared to be more resistant to infection, which could be attributed to maternal immunity from colostrum. Calves younger than 6 month old are known to be resistant to acute clinical babesiosis. At this age calves acquire innate immunity from colostrum that protects them from infections until 12 months of age. If calves get exposed to infection during this period of their life, they gain resistance against reinfection. But on the contrary, if infection was not encountered during the first year of life, then the resistance diminishes (Sandra Ríos-Tobón , Lina A. Gutiérrez-Builes 2014).

These results also provided an indication that, the parasites has been circulating within the cattle population for some time. This was confirmed by the fact that antibodies were detected in cattle above the age of twelve months, which indicates that there had been a continuous

exposure to the parasites. At that age, the maternal antibodies will have diminished and no antibodies should be detected if there was no exposure (Tembue et al. 2011; Goff et al. 2002).

On the other hand, the current study contradicted the a study carried out in Turkey b Ekici & Sevinc (2009), who found the highest prevalence of babesiosis in calves under one year old as compared to cattle aged above two years.

So, in event of controlling bovine babesiosis in the study area, one should target all age groups with more attention to adult animals. Farmers should be advised to reduce the number of male cattle to a rate of four male to one hundred females in the herd. As animals grow older, they becomes less productive but high consumers. In case of babesiosis, they also become carriers of the disease (Nair et al. 2013). Through the employment of this concept, farmers need to emphasise on removing adult animals as a control strategy.

In general, communal farmers do not practice weaning. Likewise in Ohangwena region it was also observed that calves may continue milking up to two years. However, the results of the current study contradicts some literatures, which identified that calves were found to be more susceptible to Babesiosis in comparison to adult cattle (Amorim et al. 2014; Lemma et al. 2015). The results of the current study was validated by similar results obtained from a various previous studies conducted in other countries. Abdullah-Al-Mahmud (2015) reported a higher prevalence rate in cattle aged over three years as compared to 1to 2 years of age. Adult cattle of the study area have shown to be more susceptible than calves. This is as a result of inverse age resistance to the disease (Torr et al. 2002; Muhanguzi et al. 2014).

In addition, the rapid immune responses to primary infection in calves through a complex immune mechanism might have also contributed to high prevalence in adult cattle than calves (Wikel 1996; Tembue et al. 2011). Some studies indicated that, in case of endemic instability, adult cattle might be frequently infected; while calves were still under protection of colostrum immunity (Gualito et al. 2012; Goff et al. 2002). The combination of age resistance, perhaps with maternal antibodies protection in calves, might be the contributing factor to the lower prevalence and possibly less clinical cases in calves.

The current study observed that the presence of antibodies against babesiosis increased with the age of cattle. This finding may be explained in two ways:

i) The higher occurrence observed in older cattle, may be related to the sustained tick challenge received under traditional grazing systems, with approximately more than 90% of the cattle in the study area probably exposed to tick infestation. It has been observed that where only a small number of younger animals get exposed to *Babesia* infections, a large number of adult cattle remain susceptible and would seroconvert on subsequent exposure. This situation is likely to maintain a population of susceptible individuals that could be affected by babesiosis on exposure to any of the two of *Babesia* parasites infections (Combrink et al. 2010).

ii) Under traditional cattle rearing, where milking of cows is practiced, calves are usually confined, fed at home and not allowed to graze with adults. This helps to limit the exposure of calves to ticks through the exchange of ticks between adults and calves. The only time calves are allowed to meet with cows is at the time of milking when they have to stimulate the milk let -down. This practice might possibly result in calves not only having a lower prevalence level. On the other hand, they become less resistant to babesiosis and other tick borne diseases at adult age. As the maternal immunity declines, animals may fail to seroconvert to acquire resistance in event of exposure to *Babesia* infections giving the chance of progression to clinical disease (Sergeant 2004; Cameron 2012).

Furthermore, the high prevalence in adult animals could be due to the following reasons: Calves under the age of two years remained in paddocks close to homesteads while adults grazed away far in the field. In communal grazing areas, animal met with others from different places in which some could be infested with ticks, thus facilitating the spread of ticks from one herd to another (Estrada-Peña & Salman 2013). It is a well-known fact that handling of calves for nursing is easier to farmers than handling adult animals. Therefore, it is possible that farmers in this study were able to de-tick calves rather than the adult cattle.

During the course of this study, it was discovered that there was lack of sufficient handling pens in the communal areas. This could be a challenge to the farmers limiting access to proper animal care practices such as dipping. More handling pens should be constructed in the communal areas, where farmers can de-tick animals in an attempt to curb the spread of

tick borne diseases (Schischke 2015; Gaudex 2014)**.** Animal husbandly practices might have also played the role. For example, there were more adult cattle kept on the pasture.

Chapter 6

Conclusions and recommendations

This study has served as a baseline survey to determine the prevalence of bovine babesiosis in Ohangwena region and allows the following conclusions:

The disease is prevalent throughout the region with no statistical significance between the regions, which means that cattle of both sexes at all ages were equally exposed to parasites in all constituencies of the study area. Furthermore, tick fever was found to be widely but not uniformly distributed throughout the study area.

This study has clearly identified a need for more farmer education and awareness about tick borne diseases. Effective control of this disease is important not only because of the zoonotic nature of the disease under study, but also because of its adverse impact on animal production.

The herd composition in the study area was not at recommended standard. Males were as many as females with the majority of adult cattle above five years of age.

To the best of the authors' knowledge, this study reports for the first time the prevalence of babesiosis in Ohangwena region. Based on the level of prevalence observed in this study, the area of study had not attained endemic status. Therefore since the region proved to be endemically unstable for babesiosis, a vaccination protocol to establish good herd immunity is necessary to improve production. It is also strongly recommended that similar studies in different parts of Namibia be performed and that efforts to prepare a local vaccine for the disease should be considered.

The fact that the study area has not attained endemic status means that there is a high risk of disease outbreak in case of less resistant breeds being introduced in the area. Moreover, the region offers risk for transmission of babesiosis to susceptible animals coming from areas of enzootic instability. Therefore, the use of appropriate preventive measures is needed, especially with regard to the effective control of ticks. In these circumstances, further studies are needed in order to monitor the herds for purpose of identifying factors that may pose risks to the current epidemiological status of the region.

There was no significance association between infection as the dependent variable and independent variables like sex, age and place. Therefore, this study did not prove that any of these factors were important for infection of cattle in the study area. Therefore, the control of the disease in the study area should focus all cattle irrespective of age, place and sex.

In order to determine the epidemiology of tick- borne diseases, it is necessary to understand the seasonal activities of the ticks (Ekici & Sevinc 2011). This was not established because it was beyond the scope of the present study. Therefore, future studies are needed to investigate activities of ticks over seasons of the year.

The results of this study corroborate the findings from studies conducted in countries of southern and eastern regions of Africa, where the variation of infection by *Babesia bigemina* and *Babesia bovis* depends on factors such as the age of the animal, the prevalence of vectors and measures related to their control.

Considering that the recommended bull to cow ratio under extensive grazing systems should be 1:25, the results of the current study suggest that farmers kept a large number of unproductive old age animals in the field. The incorrect bull-to-cow ratio observed in this study negatively affects the conception and fertility of the herd and so this is a hindrance to the development of the livestock industry in the study area (Rothauge 2006; Hangara et al. 2011).

The majority of cattle sampled for this study were older than five years. This high proportion of old cattle in the samples could be attributed to the fact that farmers in rural areas keep old animals until they die of old age. These cattle remain in the herd until the age when they are no longer productive, but rather increase the cost burden in terms of food and medication. Based on this, there is a need for more effort to advice farmers minimize the number of animals that are five years and older that are kept on the veld. Agricultural regulations should also include the removal of old animals to help improve productivity and farmers output.

Further research should investigate and map out the prevalence of *Babesia* parasites and other heamoparasites in each region of Namibia, in order to develop geo-referenced maps of regions as an aid for better control mechanism of tick borne diseases in Namibia.

Intervention by the government and other sectors in agriculture through farmer training and awareness campaigns is therefore recommended. The government can become involved through its field workers in the Ministry of Agriculture, Water and Forestry.

This study provides mangers in Ohangwena region with insights into knowledge they can use to improve the immunological status of herds. This information can also contribute towards the development of future interventions and manag c0076ement strategies in animal health. But this will require the generation of knowledge on the prevalence of parasites in each constituency.

Chapter 7

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Annexures

Annexure I: Ethical Approval letter

UNISA university of south africa

2013-11-29

Ref. Nr.: 2013/CAES/147

To:
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Supervisor: Mr J Oosthuizen
Department of Agriculture and Animal Health
College of Agriculture and Environmental Sciences

Dear Mr Oosthuizen and Mr Matheus

Request for Ethical approval for the following research project:

Study the prevalence of selected haemoparasites in Sanga/Nguni cattle in the flood prone area Ohangwena Region, Namibia

The application for ethical clearance in respect of the above mentioned research has been reviewed by the Research Ethics Review Committee of the College of Agriculture and Environmental Sciences, Unisa. Ethics clearance for the above mentioned project (Ref. Nr.: 2013/CAES/147) is approved after careful consideration of all documentation submitted to the CAES Ethics committee. Approval is only given for the duration of the research project.

The researcher is cautioned that any laboratories which are not accredited or incomplete are entered at own risk. Unisa will not be held liable for experimental work conducted in these laboratories.

Please be advised that the committee needs to be informed should any part of the research methodology as outlined in the Ethics application (Ref. Nr.: 2013/CAES/147), change in any way. In this instance a memo should be submitted to the Ethics Committee in which the changes are identified and fully explained.

The Ethics Committee wishes you all the best with this research undertaking.

Kind regards,

Prof E Kempen,
CAES Ethics Review Committee Chair

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Annexure II: Data collection tool (sampling form)

Babesia Samples Orbangiwena Region

	Eartag	Sex	Age	Crushpen	Result	
					B. bovis	*B. bigemina*
1.	ZC053141	M	5	Amukulungundju	N	N
2.	ZC053165	M	12	Amukulungundju	N	N
3.	ZC053535	M	4	Amukulungundju	N	1/160
4.	ZC070929	F	4	Amukulungundju	N	N
5.	ZC204412	F	8	Amukulungundju	N	N
6.	ZC204450	M	5	Amukulungundju	N	N
7.	ZC204990	F	2	Amukulungundju	N	N
8.	ZC209467	M	9	Amukulungundju	N	N
9.	ZC058072	M	2	Eembaxu	N	N
10.	ZC072414	F	2	Eembaxu	N	N
11.	ZC085411	F	4	Eembaxu	N	N
12.	ZC092435	M	8	Eembaxu	N	N
13.	ZC108889	F	5	Eembaxu	N	N
14.	ZC200464	M	7	Eembaxu	N	N
15.	ZC224928	F	6	Eembaxu	N	N
16.	ZC243825	F	8	Eembaxu	N	N
17.	ZC037878	F	8	Eemboo	N	N
18.	ZC048129	F	2	Eemboo	N	N
19.	ZC060049	F	8	Eemboo	N	N
20.	ZC092400	M	7	Eemboo	N	N
21.	ZC092484	F	11	Eemboo	N	N
22.	ZC196542	M	12	Eemboo	N	N
23.	ZC270468	M	2	Eemboo	N	N
24.	ZC279211	F	2	Eemboo	N	N
25.	04365956	F	5	Eendobe	N	N
26.	ZC139186	F	9	Eendobe	N	1/160
27.	ZC141251	M	10	Eendobe	N	N
28.	ZC141448	F	4	Eendobe	N	N
29.	ZC152233	M	4	Eendobe	N	N
30.	ZC220511	F	2	Eendobe	N	1/160
31.	ZC250853	M	7	Eendobe	N	N
32.	ZC267194	M	5	Eendobe	N	N
33.	ZC057481	F	9	Elinde	N	N
34.	ZC057830	M	14	Elinde	N	N
35.	ZC057833	M	10	Elinde	N	N
36.	ZC057917	F	4	Elinde	N	1/160
37.	ZC068348	M	12	Elinde	N	N
38.	ZC068518	F	9	Elinde	N	1/160

Annexure III: Participant consent form

i. **Participant consent form**

Participant Consent Form

BACKGROUND INFORMATION

Title and researcher(s)
The title of this research is *(*Study the prevalence of tick-borne haemoparasite in sanga/nguni cattle in living in the flood prone area of Ohangwena region, Namibia). Name of the researcher is *(Emmanuel Matheus)* from the University of South Africa, College of Agriculture and Environmental Sciences.

Reason for the research
He is studying the prevalence of tick-borne haemoparasite in sanga/nguni cattle in living in the flood prone area of Ohangwena region, Namibia. The researcher is collecting blood samples from sanga cattle to enable him to test for the presence and identification of haemoparasite at the crush pen.

Details of participation
The research involves taking blood samples from cattle through jugular or cocegeal veins and send to laboratory for diagnosis and analysis. Please feel free to ask questions now if you have any.
You will remain anonymous therefore there is no need to provide your name.

CONSENT STATEMENT

1. I understand that my participation is voluntary and that I may withdraw from the research at any time, without giving any reason.

2. I am aware of what my participation will involve.

3. I understand that there are no risks involved in the participation of this study. [Or the risks in this study are that I might become upset recalling memories of tick-borne diseases I experienced].

4. All questions that I have about the research have been satisfactorily answered.

I agree to participate.

Participant's signature: _____

Date: _____

Constituency _____ Village_____

Annexure IV: Permission letter from the Department of Agriculture and Animal Health

UNISA university of south africa

2013 September 16

To whom it may concern

CONFIRMATION OF PROPOSAL PRESENTATION AT THE DEPARTMENT AGRICULTURE AND ANIMAL HEALTH by Mr Emmanuel Matheus (35209488)

I confirm that the above student **Mr Emmanuel Matheus (35209488)** presented the research proposal "Prevalence of selected Haemoparasites in Sanga/Nguni cattle in the flood prone area Ohangwena region, Namibia" at the Department of Agriculture and Animal Health on 12 September 2013. He has also effected changes as suggested by academic staff members and we therefore suggest that he proceeds with ethics clearance and other administrative matters.

Yours sincerely

Kbatha

[Electronic signature]

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Annexure V: Article under by "Indian Journal of Animal Research"

Prevalence of babesiosis in Sanga cattle in the Ohangwena region of Namibia

[1]EMMANUEL KAMUTYATSHA MATHEUS; [1]JOHAN OOSTHUIZEN; [1]CHRISTIAN ANAYOCHUKWU MBAJIORGU [1*]JAMES WABWIRE OGUTTU

[1]Department of Agriculture and Environmental Sciences, University of South Africa, Johannesburg, South Africa

Running/short title: Prevalence of babesiosis in Sanga cattle

Abstract

A total of 392 samples were randomly collected. The indirect fluorescent antibody (IFA) test was used to test for antibodies against *Babesia*. The herds had almost equal numbers of males (49%) and females (51%). Cattle ≥ 5 years constituted 63% of the sampled animals, while cattle 0-2 years old were in the minority (14%). *Babesia bigemina* had the highest estimated prevalence at 36.5%. Mixed infections had an estimated prevalence of 13.2%. Based on age, 3 to 4 years old had the highest prevalence of both *Babesia bovis* (23.9%) and *B. bigemina* (44.6 %). The 0 to 2 years of age category had the lowest prevalence of both *B. bovis* (12.3%) and *B. bigemina* (29.8 %). With co-infections, the 3-to-4-year-olds also had the highest prevalence (18.5 %), while 0-to-2-year-olds had the lowest prevalence (8.8%). It was observed that *B. bigemina* had the high prevalence. The region was endemically unstable.

Key words: Babesiosis; prevalence; distribution; vectors; ticks; B*abesia*; zoonotic